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SYMPOSIUM

Chronic Fungus Infections

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FUNGUS INFECTIONS OF THE HAIR AND NAILS

NORMAN F. CONANT, PH.D.

DURHAM, N. C.

From the School of Medicine, Duke University

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FUNGUS infections of the hair may be divided clinically into three categories, based on the type of infecting organism: (1) trichomycosis caused by *Corynebacterium tenuis*, (2) piedra caused by *Piedraia hortai* and *Trichosporon beigelii*, and (3) ringworm of the scalp (tinea capitis) caused by dermatophytes of the genera Microsporum and Trichophyton. Fungus infections of the nails, tinea unguium, or onychomycosis, appear clinically identical regardless of the fungus etiology, which may be one of several species of Trichophyton, *Epidermophyton floccosum*, and, very rarely, *Microsporum canis*.

TRICHOMYCOSIS

Trichomycosis is an asymptomatic nondestructive infection of the axillary and pubic hairs characterized by continuous sheaths or isolated soft adherent nodes on the hair shafts. The growth surrounding the hairs is essentially a yellowish colony of *Corynebacterium tenuis* which had previously been classified as a fungus belonging to the genus Nocardia.[1] The nodules may also be red or black if the bacterial growth contains associated chromogenic cocci.

The hairs appear lusterless, but undamaged, and the hair follicle and surrounding skin is not involved (Fig. 1,*A*). A diagnosis is based on the microscopic appearance of the nodules or sheaths in potassium hydroxide preparations where they are seen to be clusters of bacteria, bacilli, and associated cocci (Fig. 1,*B*).

Treatment consists of shaving and the use of soaps containing antibacterial substances to prevent recurrence.

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PIEDRA

Piedra is a true fungus infection of the hair of the scalp, beard, and mustache characterized by stony hard nodules along the hair shaft. Black piedra, caused by *Piedraia hortai*, is an infection of the hair of the scalp distinguished by hard, brown-to-black, adherent nodules on the hair shaft. The disease is endemic in several countries in South America, Java, and Cochin-China.[2] White

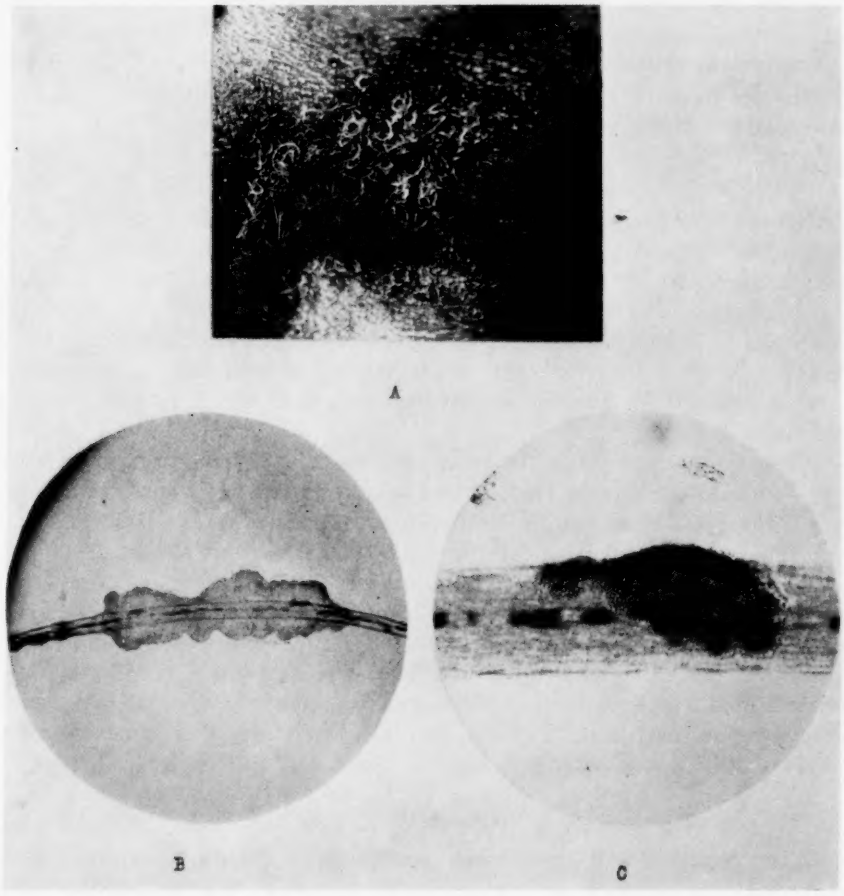

Fig. 1.— *A*, Trichomycosis. Gross appearance of hairs surrounded by bacterial sheaths of *Corynebacterium tenuis*. *B*, Trichomycosis. Microscopic appearance of bacterial sheath on hair shaft. *C*, Piedra. Black nodule of *P. hortai* on hair shaft. (From Conant, N. F., et al.: Manual of Clinical mycology, W. B. Saunders Co., 1954.)

piedra caused by *Trichosporon beigelii*, is an infection of the hairs of the scalp, beard, and mustache distinguished by hard, whitish adherent nodules on the hair. This variety of piedra is endemic in several South American countries and has also been reported from Europe.[3]

In "black piedra," the fungus invades beneath the cuticle and develops a mass of brownish hyphae around the hair shaft to form a hard nodule (Fig. 1,*C*).

The hairs do not break off, remain essentially undamaged, and the hair follicle and surrounding skin is not involved. In "white piedra," the involved hairs may resemble trichorrhexis nodosa because of the nodular appearance and frequent destruction of the hair shaft. With one exception piedra has been encountered in the United States only in natives from the areas mentioned above.[4]

Diagnosis is made on the microscopic appearance of the nodules in potassium hydroxide preparations of the affected hairs. Such preparations reveal the nodules to be a mass of wide, closely septate hyphae which distinguishes them from the bacterial mass seen in trichomycosis.

Treatment consists of cutting, clipping, or shaving the hairs from the affected area followed by daily shampooing of the scalp and the application of an antifungal ointment to the scalp or skin of the bearded region.

RINGWORM OF THE SCALP

Ringworm of the scalp or tinea capitis is a fungus infection seen particularly in children, less frequently in adults, and caused by species of dermatophytes of the genera Microsporum and Trichophyton. In the United States prior to 1942-1943, the disease occurred sporadically among children in thickly settled urban areas. At this time, however, it became evident that hundreds of children in urban areas in several sections of the country were infected with *Microsporum audouini* and that the disease had assumed the characteristics of an epidemic.[5-7]

Whereas *M. audouini* is the recognized cause of epidemic ringworm of the scalp in the United States, *Trichophyton tonsurans* was reported to be the predominant cause of tinea capitis in Mexico and Puerto Rico.[8,9] Unlike *M. audouini*, this fungus not only infects the scalp of children but also infects that of adolescents and adults. Recently, an increased incidence of infection by *T. tonsurans* in the southwestern and northeastern United States has been associated with immigration of Mexicans into the southwestern states and of Puerto Ricans into the New York area.[10-13] At the present time, therefore, fungus infections of the scalp in both children and adults have become so prevalent in certain areas of the United States as to cause these infections to be considered of major public health significance.

MICROSPOROSIS

Tinea capitis caused by *Microsporum audouini*, *M. canis*, and *M. gypseum* is characterized by scaly, erythematous lesions which enlarge peripherally to form grayish areas of alopecia in which the infected hairs are broken off a few millimeters above the surface of the scalp.

M. audouini, a species found only in man, causes epidemic tinea capitis in children by direct transmission from person to person. This species usually causes noninflammatory chronic infections which show little tendency to undergo spontaneous involution (Fig. 2,*A*). *M. canis* and *M. gypseum*, species infecting animals, cause sporadic tinea capitis by occasional transmission from animal to man. These species usually cause acute inflammatory lesions which may result in kerion formation (Fig. 2,*B*). As a result of the inflammatory re-

action these infections show a marked tendency to undergo spontaneous involution.

The diagnosis of tinea capitis, caused by species of Microsporum, is suggested by the appearance of the grayish scaly areas of alopecia, "gray patch

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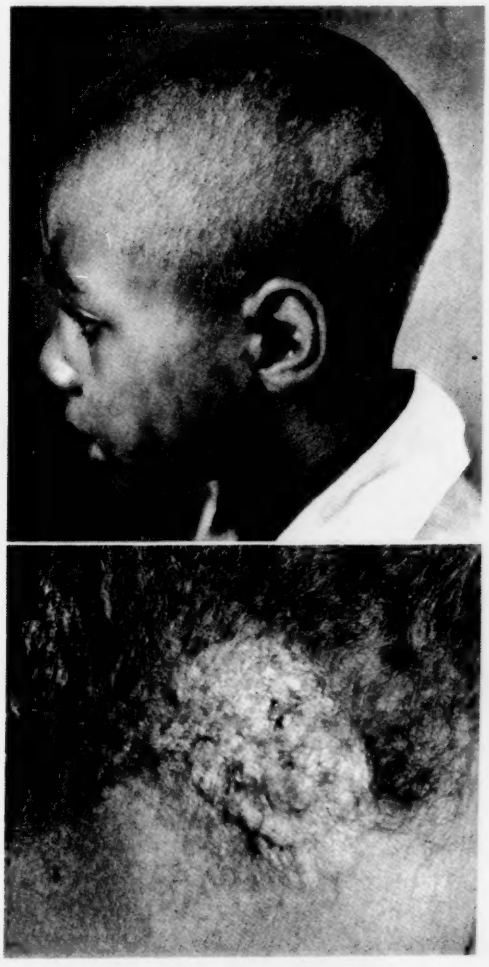
A.



B.

Fig. 2.—*A*, Noninflammatory tinea capitis, gray patch ringworm, caused by *Microsporum audouini*. *B*, Inflammatory tinea capitis, kerion, caused by *Microsporum canis*.

ringworm." It is confirmed by the fluorescence of infected hairs under the Wood's light, by the microscopic appearance of infected hairs in potassium hydroxide preparations and by culture.[14] Hairs infected with *M. audouini* and *M. canis* show a bright greenish fluorescence with Wood's light. Hairs

infected with all three species show an ectothrix arrangement of small spores in a sheath surrounding the hair shaft. Since hairs infected with all three species of Microsporum have the same microscopic appearance, species identification can be made only by culture (Fig. 3).

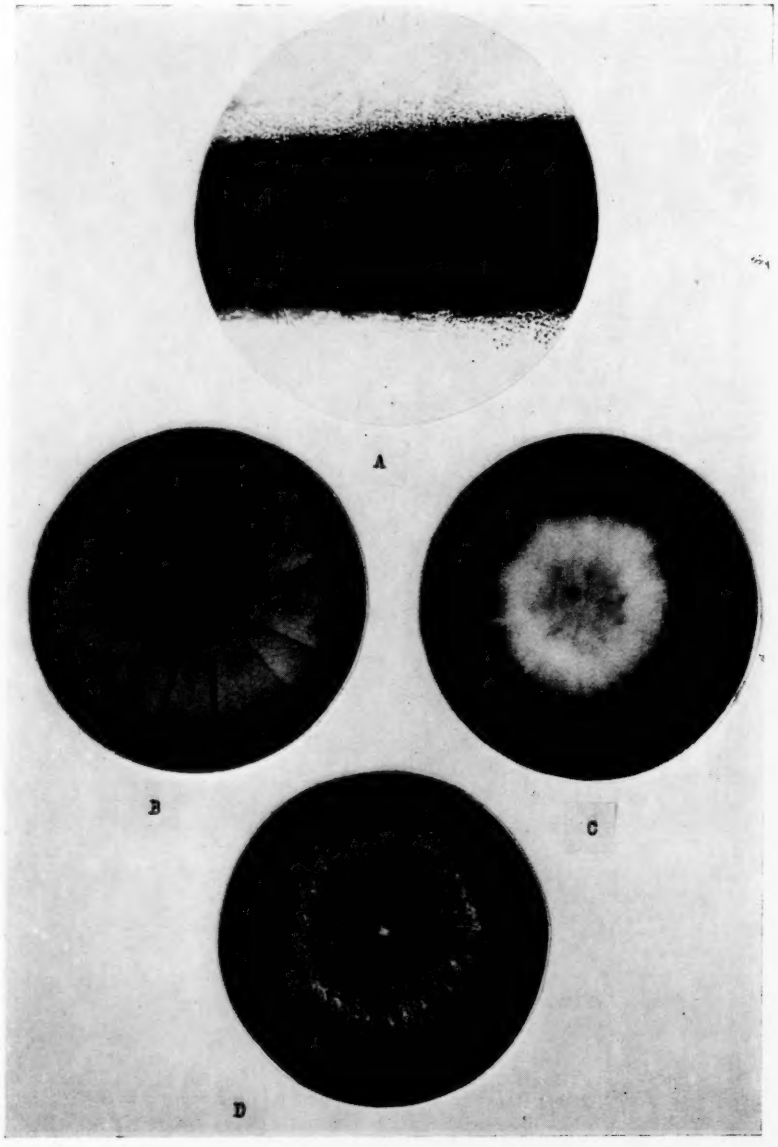


Fig. 3.—*A*, Microsporum (ectothrix) hair with sheath of small spores seen in KOH preparation. *B*, Culture of *M audouini* on Sabouraud's glucose agar. *C*, Culture of *M. canis* on Sabouraud's glucose agar. (*B* and *C*, From Conant et al., W. B. Saunders Co.) *D*, Culture of *M. gypseum* on Sabouraud's glucose agar, (From Smith, David T., and Conant, Norman F.: Zinsser's Textbook of Bacteriology, 10th edition. Courtesy of Appleton-Century-Crofts, Inc.)

Successful treatment of tinea capitis depends upon removal of the infected hairs. The presence of the spore sheath which surrounds the hair and extends downward into the hair follicle as well as the presence of hyphae extending to Adamson's fringe within the hair shaft itself are predominant factors which prevent a desirable curative action of antifungal substances or successful manual epilation of the entire hair. Antifungal substances cannot penetrate the hair follicle to destroy the spore sheath or kill the intrapilary hyphae. Manual epilation usually breaks off the hair above Adamson's fringe leaving a residual infection which progresses with the newly growing hair.[15]

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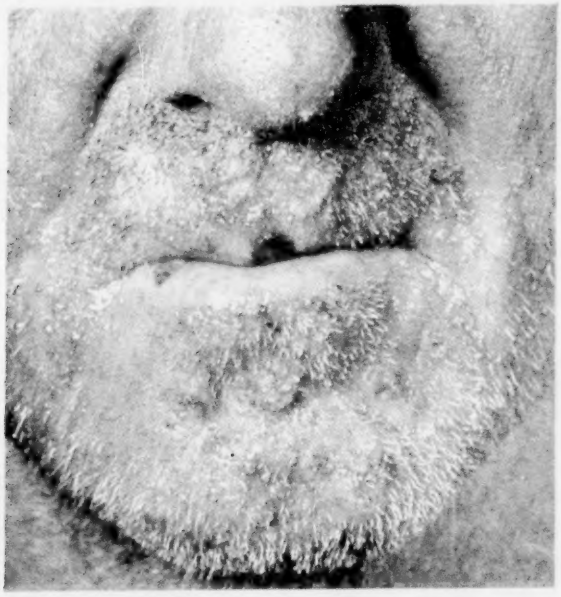

Fig. 4.—Tinea barbae Caused by *Trichophyton mentagrophytes*.

In noninflammatory infections removal of the infected hairs is best accomplished by x-ray epilation. Before resorting to x-ray epilation, however, the infection should be observed closely for a period of three to four months, during which prophylatic and curative measures should be tried. The hair should be clipped, infected areas of the scalp outlined with the aid of a Wood's light, and a treatment regimen carefully explained to the parent. This treatment might consist of daily shampoos followed by an application of any one of several ointments; Furaspor, Desenex, Sopronol, or a 3 to 5 per cent ammoniated mercury ointment. The hairs within the affected areas should be manually epilated once a week. The head should be covered at all times, day and night, with a tight-fitting cotton skull cap which should be frequently boiled and washed.

Since spontaneous epilation follows an inflammatory reaction and x-ray epilation is not necessary, the individual lesions should be observed closely for signs of an acute response to the infection. Such reactions may be minimal or

progress to kerion formation. In such cases the scalp is treated daily with warm compresses and every effort should be made to prevent secondary bacterial infection. The occurrence of pus in the boggy "kerion" lesion is due to the allergic reaction of the tissues and is, therefore, not necessarily indicative of an associated bacterial infection. When secondary bacterial infection does occur, however, topical and/or systemic antibacterial therapy should be used.

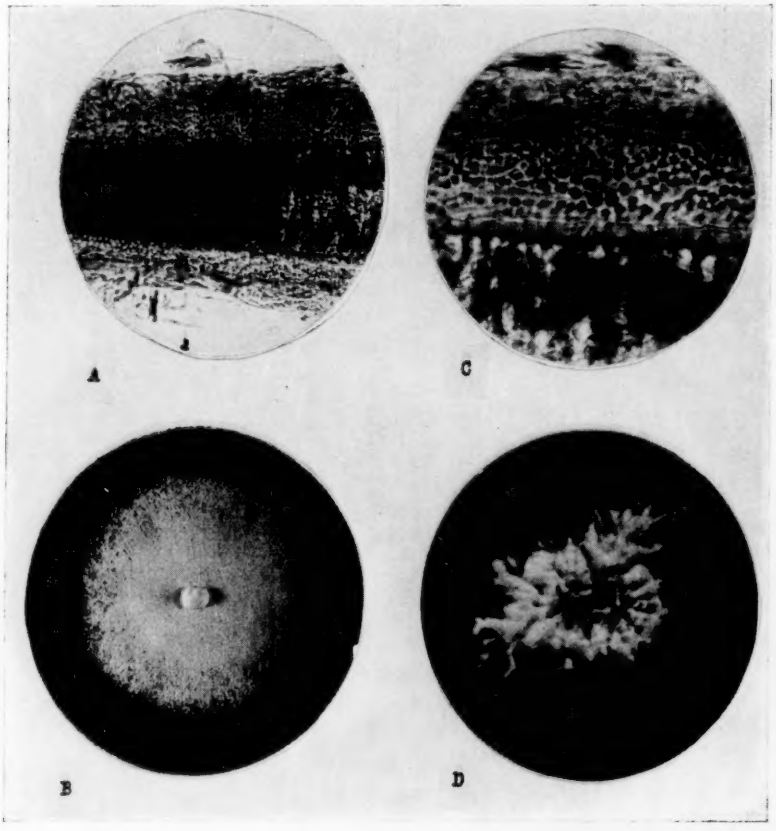

Fig. 5.—*A*, *Trichophyton mentagrophytes* (ectothrix) hair with chains of arthrospores seen in KOH preparation. (From The Merck Report **64:**21, 1955, Fig. 3a.) *B*, Granular culture of *T. mentagrophytes* on Sabouraud's glucose agar. (From Conant et al., W. B. Saunders Co.) *C*, *Trichophyton verrucosum* (ectothrix) hair with chains of arthrospores seen in KOH preparations. (From the Merck Report **64:**21 1955, Fig. 6c.) *D*, Slow growing folded colony of *T. verrucosum* on Sabouraud's glucose agar. (From Conant et al., W. B. Saunders Co.)

TRICHOPHYTOSIS

Species of Trichophyton cause infection of the scalp in children, adolescents, and adults and of the bearded area in man. Tinea capitis, "black dot" ringworm, tinea barbae, and favus are terms used for the variety of clinical conditions caused by these fungi. For the most part, infections caused by species

of Trichophyton in man are sporadic due to contact with infected animals (dog, cat, horse, cow, etc.). Infections caused by the anthropophilic species, however, may involve an entire family, although widespread epidemics (such as caused by *M. audouini*) are not known. The danger exists, however, that infections caused by *T. tonsurans* may assume epidemic proportions in certain areas of the United States.

TINEA CAPITIS—TINEA BARBAE

T. mentagrophytes, *T. verrucosum*, *T. tonsurans*, *T. violaceum*, and *T. schoenleini* cause ringworm of the scalp in adults as well as in children. *T. mentagrophytes* and *T. verrucosum* are zoophilic species which cause acute inflammatory (suppurative) ringworm, tinea capitis, and tinea barbae, with extreme kerion

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Fig. 6.—Tinea capitis, black dot ringworm, caused by *Trichophyton violaceum*.

formation (Fig. 4). Such infections must be differentiated from pyoderma, folliculitis decalvans, and impetigo by finding the fungi on microscopic examination of the hair. Invasion of the hair by these fungi is of the ectothrix type with chains of arthrospores outside of the hair shaft. The type of invading fungus is determined by culture (Fig. 5). The treatment is that previously suggested for inflammatory ringworm infections.

Tinea capitis caused by species of Trichophyton result in a scaly patchy alopecia with thining of the hair in affected areas rather than discrete grayish areas of alopecia characteristic of microsporosis. Infection by *T. tonsurans* and *T. violaceum* result in the so-called "black dot" ringworm (Fig. 6). The hairs usually break off at the surface of the scalp leaving dark stubs embedded in the scaly areas. Infection by *T. schoenleini* results in yellowish, cup-shaped, elevated crusts (scutula) characteristic of favus.

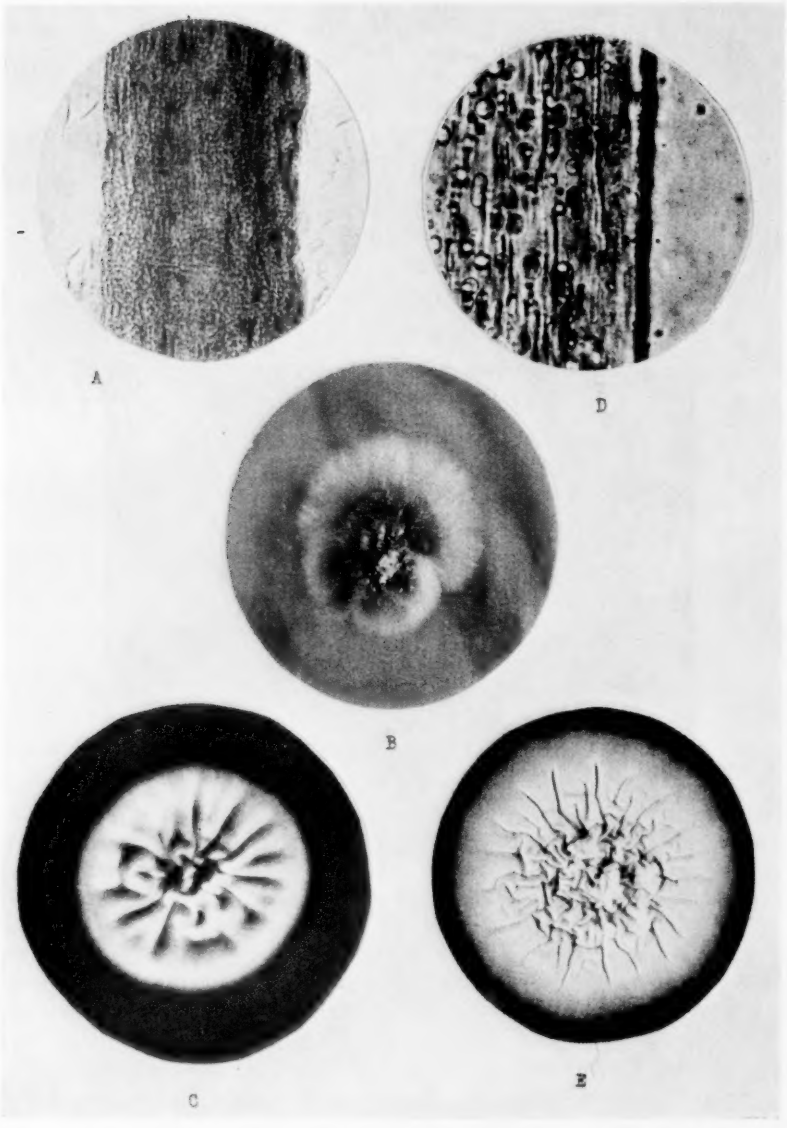

Fig. 7.—*A*, Endothrix hair (*T. violaceum* and *T. tonsurans*) with chains of arthrospores inside of hair shaft seen in KOH preparations. (From Conant et al., W. B. Saunders Co.) *B*, Culture of *T. violaceum* on Sabouraud's glucose agar. (From The Merck Report **64**:22, 1955, Fig. 5a.) *C*, Culture of *T. tonsurans* on Sabouraud's glucose agar. (From Conant et al., W. B. Saunders Co.) *D*, Endothrix (favus) hair with tunnel and bubble formation inside hair shaft characteristic of infection by *T. schoenleini*. (From The Merck Report **64**:22, 1955, Fig. 5a.) *E*, Culture of *T. schoenleini* on Sabouraud's glucose agar. (From Conant et al., W. B. Saunders Co.)

T. tonsurans, *T. violaceum*, and *T. schoenleini* are anthropophilic species which cause chronic infections, frequently of many years' duration. The chronicity of these infections and the variety of clinical manifestations make them difficult to differentiate from psoriasis, seborrheic dermatitis, bacterial folliculitis, cicatricial alopecia, impetigo, and lupus erythematosus. Since hairs infected with these fungi do not fluoresce, microscopic examination of potassium hydroxide preparations of several selected hairs may be necessary before the fungus nature of the infection is established. Invasion of the hair by *T. tonsurans* and *T. violaceum* is of the endothrix type with mycelium and arthrospores filling and crowding the interior of the hair, but the "favus" endothrix hair has a characteristic appearance (Fig. 7,*A*). A few hyphae may be seen inside the hair but the typical appearance is that of a hair that has been invaded by a fungus, with subsequent degeneration of the fungus, leaving digested areas that resemble tunnels or bubbles of various sizes (Fig. 7,*D*).

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Unless an inflammatory response occurs, diseases caused by the endothrix Trichophyton are best treated with x-ray. Following x-ray treatment the scalp should be treated vigorously with antifungal ointments and residual hairs removed by manual epilation.

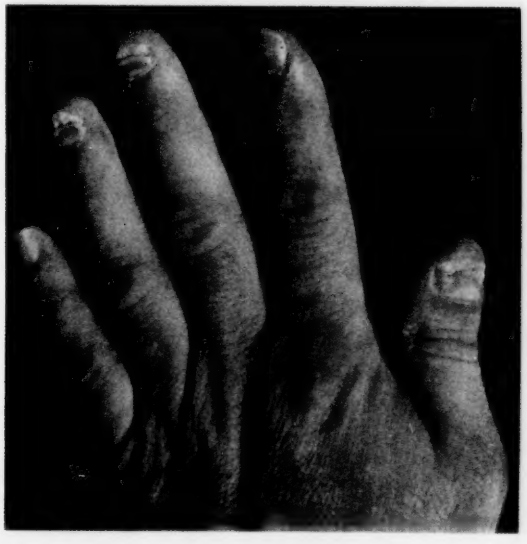

Fig. 8.—Onychomycosis, tinea unguium, caused by *Trichophyton rubrum*.

TINEA UNGUIUM

Fungus infections of the nails, onychomycosis, may be caused by many species of dermatophytes but *T. rubrum* and *T. mentagrophytes* are the usual etiologic agents. Other species of Trichophyton, *Epidermophyton floccosum*, and *Microsporum canis* may also infect the nails. Toenails are involved more frequently than fingernails, and it is unusual to have all nails involved simultaneously (Fig. 8).

Infection usually takes place at the lateral edge of the nail with progression beneath the nail plate to involve the keratin of the nail bed. Widespread involvement of the nail bed eventually causes an accumulation of keratinous detritus beneath the nail plate and subsequent invasion and destruction of the nail plate.

A diagnosis of onychomycosis is made by finding hyphal fragments in a microscopic examination of potassium hydroxide preparations of the debris beneath the nail (Fig. 9,*A*). All of the fungi which can infect the nails appear identical in such preparations. Cultures are necessary to identify the particular fungus in a given infection (Fig. 9,*B* and *C*).

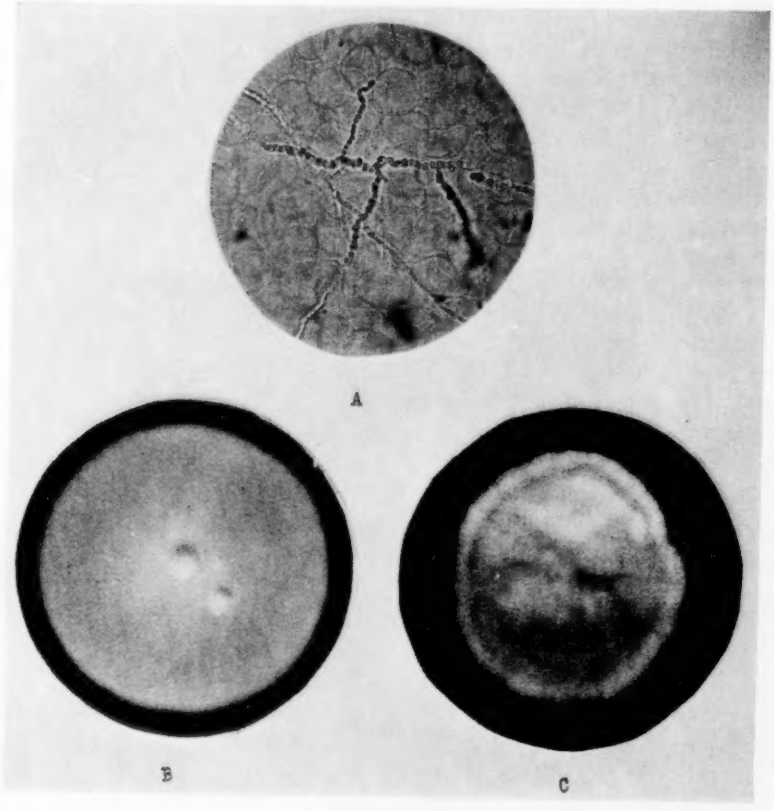

Fig. 9.—*A*, Branching hyphae seen in KOH preparation of scrapings of infected nail. *B*, Culture of *T. mentagrophytes* on Sabouraud's glucose agar. *C*, Culture of *T. rubrum* on Sabouraud's glucose agar. (*B* and *C*, From Conant et al., W. B. Saunders Co.)

There is no satisfactory treatment for onychomycosis. Surgical avulsion usually is not recommended. A discolored, friable, lusterless nail may be removed as much as possible by cutting, clipping, and filing. Daily soaking or the use of a keratolytic agent to soften the nail will aid in its removal by mechanical methods. An antifungal preparation may be applied to the nail but it is doubtful that it will penetrate the keratinous material in which the fungus is embedded.

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SUPERFICIAL MYCOTIC INFECTIONS OF THE SKIN: DIAGNOSIS AND THERAPY

J. LAMAR CALLAWAY, M.D., AND SIDNEY OLANSKY, M.D.

DURHAM, N. C.

From the School of Medicine, Duke University

(Received for publication Nov. 26, 1956.)

SUPERFICIAL fungi are widespread and stubborn pathogens. The diseases they produce are not fatal but are frequently disabling. During World War II, dermatophytosis was the second most common cause of admission to the hospital for dermatologic disease among men and women in the Armed Forces, eczema being the first.

Classification of the superficial fungi on an etiologic basis is unsatisfactory, as a single species may cause a variety of clinical manifestations in different parts of the body or in different individuals. On the other hand, the same clinical picture may be produced by different species of fungi. In the following discussion, fungus infections will be considered according to the topography of the body involved.

TINEA PEDIS (DERMATOPHYTOSIS; "ATHLETE'S FOOT")

Tinea pedis is perhaps the most common form of superficial fungus infection. Surveys in student groups and the Armed Forces have shown a varying incidence, rising as high as 65 per cent. The common organisms found are *Epidermophyton floccosum*, various species of Trichophyton, and rarely species of Microsporum and *Candida albicans*.

Symptomatology.—Four clinical forms of tinea pedis will be described.

1. *Chronic hyperkeratotic form:* This type is characterized by thickening and absence of vesicles. The eruption is often limited to the soles and sides of the feet. Its appearance is usually that of scales and hyperkeratosis on a red base. This type is usually asymptomatic and *Trichophyton rubrum* is often the etiologic agent.

2. *Chronic intertriginous form:* (Fig. 1.) The only clinical sign may be fissuring of the toe webs, especially the webs between the fourth and fifth toes. More often the skin of the interspaces of the toes appears white, macerated, and soggy. Hyperhidrosis is almost invariably present. The so-called "soft corn" often proves to be a fungus infection.

3. *Subacute form:* (Fig. 2.) This form is characterized by the presence of vesicles or vesicopustules and may extend onto the dorsum of the foot or the sole of the foot. The fluid from the vesicles is sticky and clear unless infection occurs.

The latter is not uncommon and may lead to cellulitis, lymphangitis, and lymphadenitis.

Fig. 1.—Chronic intertriginous dermatophytosis.

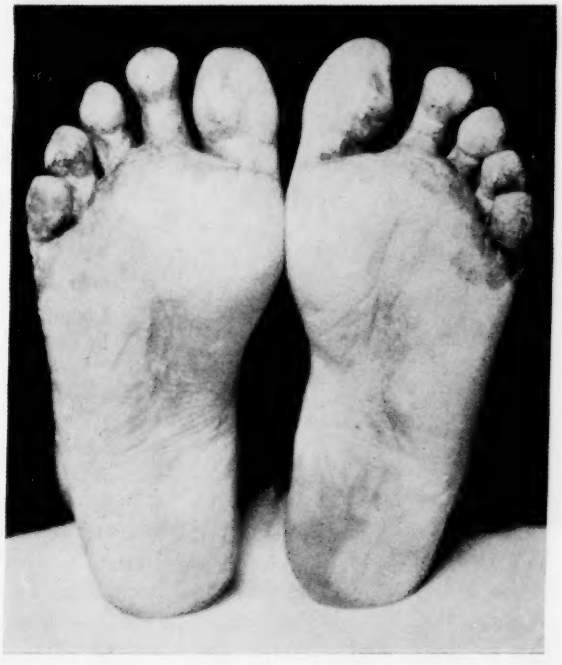

Fig. 2.—Subacute intertriginous dermatophytosis.

4. *Acute form:* (Fig. 3.) This form is more acute than the previously described form and is commonly associated with secondary infection. It may produce a clinical syndrome resembling erysipelas of the leg. Rupture of the vesicles and pustules may produce ulceration.

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Epidemiology.—The epidemiology of "athlete's foot" is not clear. Recently Baer and his associates[1,2] have demonstrated that pathogenic fungi are widespread and that many or most people are exposed to them. In addition, deliberate attempts to infect nonsusceptible individuals were not successful. It would seem therefore that host factors determine whether or not infection occurs. Vascular disease of the extremities and hyperhidrosis seem to be important determinants in the susceptibility of people to fungus infection of the feet.

Diagnosis.—The diagnosis of fungus infection can be made only by demonstrating pathogenic fungi in the lesion. This can be accomplished by cultural and/or potassium hydroxide examination, described elsewhere in this Symposium.

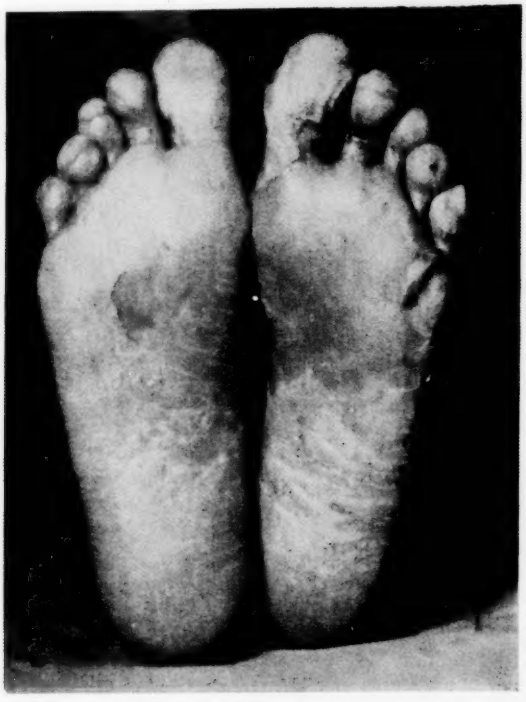

Fig. 3.—Acute intertriginous dermatophytosis.

Treatment.—Dry keratotic or chronic intertriginous lesions are best treated by foot soaks for thirty minutes each night. Satisfactory preparations for these soaks are potassium permanganate solution (1:4,000 or 1:8,000), or prophyllin soultion. After the soaks, Castellani's (carbolfuchsin) paint, Verdefam, one-half strength Whitfield's ointment, Pragmatar ointment, or Desenex, Sopronol, or Asterol ointment may be applied. A dusting power such as Desenex, Sopronol, or Asterol should be applied in the morning. Treatment should be continued for one week after lesions are no longer visible and the dusting powder should be continued indefinitely as a prophylactic measure. If the etiologic agent is *Trichophyton rubrum*, Verdefam is the most effective remedy.

Acute and infected tinea pedis should be managed by bed rest or modified bed rest and elevation of the feet. Continuous compresses of potassium permanganate solution (1:4,000), Burow's solution (1:40), prophyllin solution, or silver nitrate (¼ per cent) are very effective. Mechanical débridement rather than keratolytics should be used and the vesicles and pustules should be clipped. Systemic antibiotics may be necessary to control secondary infection. When the acute lesions subside, management is similar to that described for the chronic forms.

DERMATOPHYTIDS ("IDS")

Dermatophytids are secondary eruptions occurring in specifically sensitized individuals by hematogenous spread of fungi or their products from a primary focus. The criteria for an "id" are: (1) demonstration of a pathogenic fungus at primary site; (2) no pathogenic fungi in the secondary "id" lesions; (3) a positive intradermal Trichophyton skin test; (4) the "id" disappears without specific treatment when the primary focus is eradicated.

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Fig. 4.—Dermatophytid of the hands.

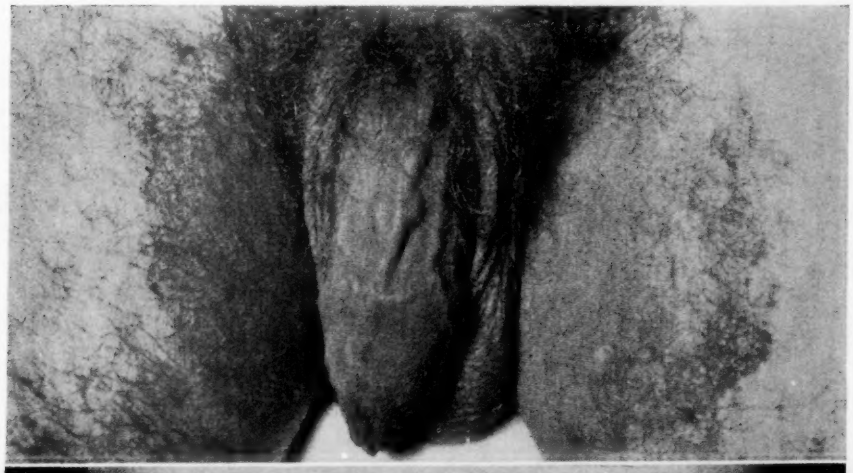
The common sites for "ids" are the sides of the fingers and hands (Fig. 4) but they may become generalized. The "id" lesions are usually deep-seated vesicles that may take other forms. Recently it has been demonstrated that some cases of erythema annulare centrifugum may be "ids."[3,4]

The basic treatment of "id" consists of treating the primary focus. This should be conservative to avoid exacerbating the "id." The "id" lesions are treated symptomatically.

TINEA CRURIS ("JOCKEY ITCH")

Tinea cruris is a fungus infection involving the groin, perineum, and perianal region (Figs. 5 and 6). The common organisms are *Epidermophyton floccosum* and species of Trichophyton. It is characterized by well-marginated areas of

Fig. 5.



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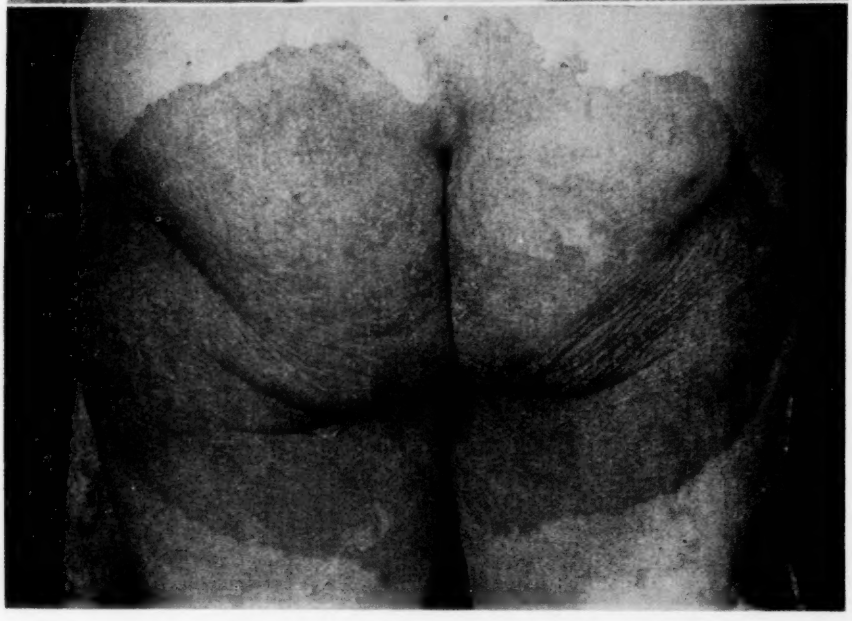

Fig. 5.—Tinea cruris.

Fig. 6.—Tinea cruris involving the buttocks.

brownish-red scaly dermatitis bordered by minute vesicles or vesiculopustules. Itching is often severe during hot weather but may be absent in cool weather.

The treatment is similar to that of tinea pedis but ointments are poorly tolerated in intertriginous areas. Potassium permanganate (1:4,000) sitz baths twice daily followed by Castellani's paint and dusting powder is often effective. If the organism is *Trichophyton rubrum*, Verdefam applied to the lesions twice daily is often curative.

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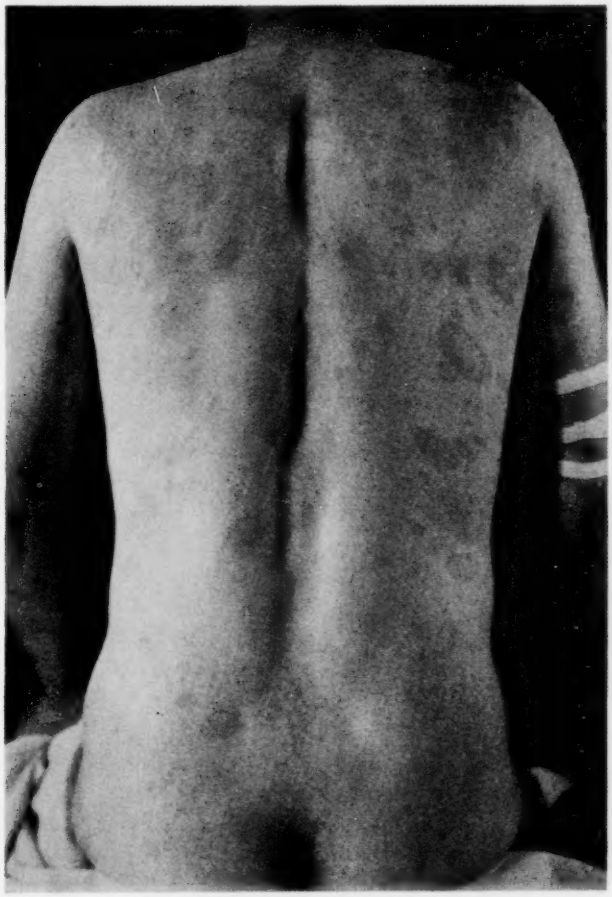

Fig. 7.—Tinea corporis.

TINEA CORPORIS (TINEA GLABROSA)

Tinea corporis (Fig. 7) may be caused by many species of Microsporum and Trichophyton and may produce lesions that vary from simple scaling to granulomas. The typical lesion is annular and usually tends to heal in the center. The periphery is marked by vesicles. The lesions may coalesce to form large plaques.

Itching varies but is often severe in warm weather. *Trichophyton rubrum* has recently been described as producing deep nodular perifollicular lesions of the legs.[5,6]

Treatment.—The lesions may be scrubbed with soap and water twice daily. Then Pragmatar ointment, ½ strength Whitfield's ointment, 3 per cent precipitated sulfur, and 3 per cent salicylic acid in aquaphor, Castellani's paint, Verdefam, or similar desquamating ointments may be used.

ERYTHRASMA

Erythrasma is a superficial infection caused by *Nocardia minutissima* (Fig. 8). The disease is asymptomatic and affects only the stratum corneum of the axillae, the groin, and occasionally other intertrigenous areas. It is characterized by

Fig. 8.—Erythrasma of the groin.

small scaly patches which coalesce into well-defined irregular patches. The patches are brown to brownish-red in color. Itching is minimal and the disease is often discovered on routine physical examinations.

Treatment.—Scrub the lesions twice daily with soap and water and then apply sodium hyposulfite solution (20 per cent), Castellani's paint, or Verdefam twice daily. The disease should be treated for about two weeks after all lesions have disappeared.

TINEA VERSICOLOR

Tinea versicolor is an infection of the smooth skin caused by *Malassezia furfur* (Fig. 9). Hyperhidrosis seems to be a predisposing factor. Lesions are most commonly seen on the trunk but may be seen on the neck and face. They

are irregular fawn colored scaly patches resembling erythrasma but lighter in color. These lesions screen out the tanning rays of the sun and the involved areas do not tan. In the summer, the uninvolved skin becomes dark, and the involved areas remain light (pseudoachromia). The lesions are usually asymptomatic but occasionally itching is complained of by the patient.

Diagnosis.—The area is cleaned with xylol or ether and allowed to dry. Scales are then scraped from the lesion onto a glass slide. A drop of 10 per cent potassium hydroxide is added and a cover slip is applied. The slide is gently heated. The fungus is seen as clusters of thick-walled round forms surrounded by short fragments of mycelium. This fungus cannot be cultured.

The treatment of tinea versicolor is like that of erythrasma.

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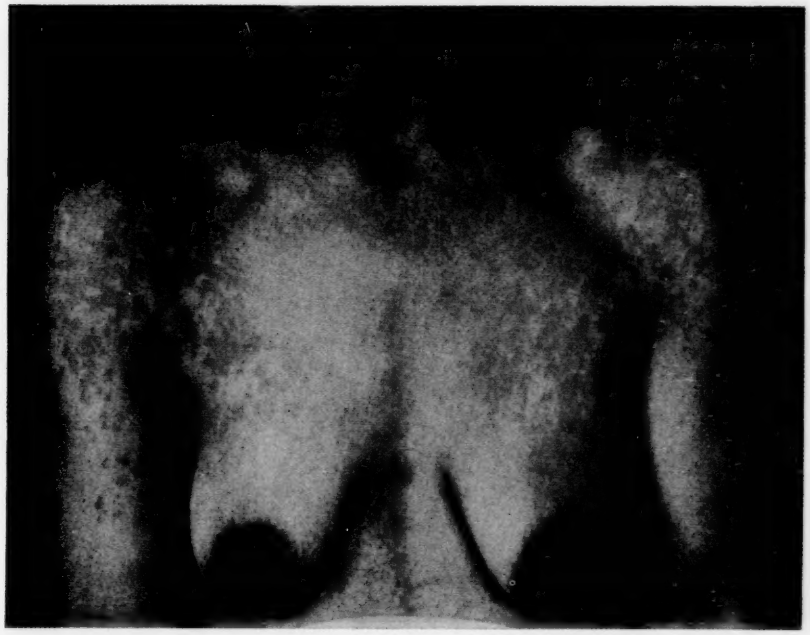

Fig. 9.—Tinea versicolor.

TRICHOMYCOSIS AXILLARIS

Trichomycosis axillaris is an infection of the axillary or pubic hair by *Corynebacterium tenuis* or *Nocardia tenuis*. Concretions, black, yellow, or red form on the hair in beadlike nodules or as a continuous sheath. The hair appears lusterless and brittle and breaks easily. The infection is confined to the shaft of the hair. It does not extend to the roots or to the surrounding skin.

Diagnosis.—Infected hairs are placed on a slide in a 10 per cent solution of potassium hydroxide. A cover slide is added, and the slide may be gently heated for clearing. The concretions can be seen surrounding the hair shaft.

Treatment.—The axillary or pubic hair should be shaved and followed by the daily application of 2 per cent formalin or 1 per cent bichloride of mercury in 70 per cent alcohol or 3 per cent sulfur ointment.

OTITIS EXTERNA (OTOMYCOSIS)

Otitis externa (Fig. 10) is a subacute or chronic infection of the external ear and ear canal from which a number of bacteria and rarely pathogenic fungi can be isolated, the etiologic significance of which is not clear. Excessive heat and moisture are predisposing. The patient complains of a sense of fullness and itching in the ears. The canal is edematous, erythematous, and crusted.

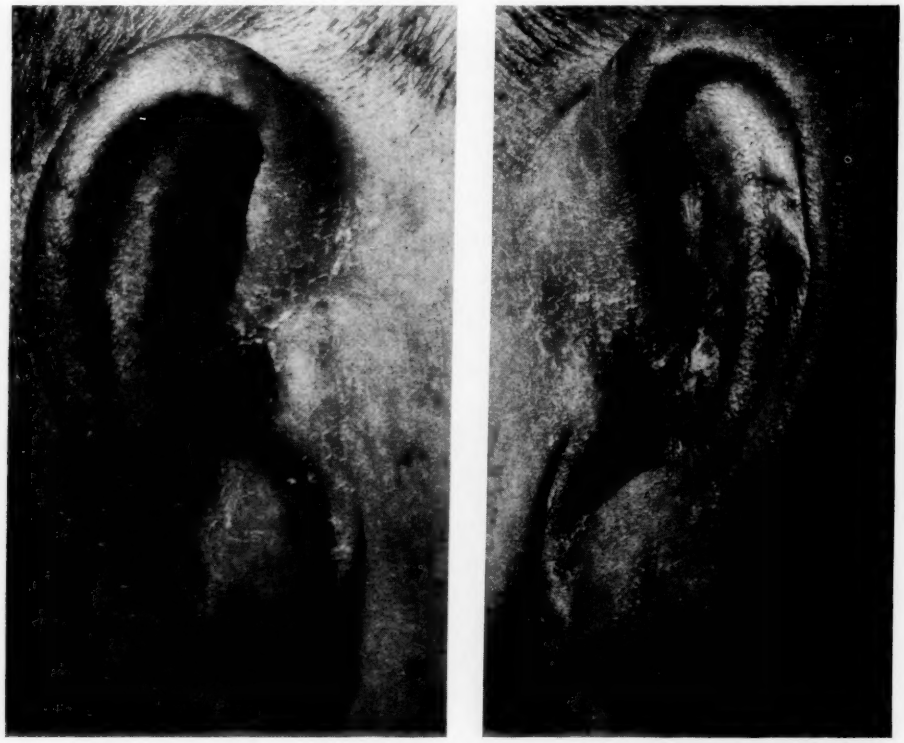

Fig. 10.—Otitis externa.

Treatment.—Acute infections should be treated conservatively to reduce swelling and permit adequate cleansing of the canal. This may be accomplished by inserting a cotton wick saturated with Burow's solution, 5 per cent aluminum acetate solution, or a urea-acetic acid solution. This should be followed daily by cleaning with swabs or irrigation with hypertonic saline. Swimming must be prohibited. In the chronic or subacute infections, antibiotics (e.g., chloramphenicol, bacitracin, Aureomycin, Terramycin, etc.) in otic form may be used with beneficial results.

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MISCELLANEOUS FUNGUS DISEASES

DAVID T. SMITH, M.D.*

DURHAM, N. C.

From the School of Medicine, Duke University

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A BRIEF review of sporotrichosis, geotrichosis, aspergillosis, penicilliosis, mucormycosis, chromoblastomycosis, maduromycosis, and rhinosporidiosis will be presented in this article. Sporotrichosis ranks with the major fungus infections of man and deserves, if space permitted, a more comprehensive presentation. The other mycoses listed above are not of major importance in this country.

SPOROTRICHOSIS

Sporotrichum schenckii occurs in all parts of the world and has been isolated from various types of organic materials. The infection occurs most frequently in farmers, laborers,[6] and horticulturists. One epidemic involving 2,825 laborers in the gold mines of South Africa was traced to infected timbers used in the mines.[8]

The organism occurs in the tissues of infected man and experimentally infected animals as a small oval or fusiform body (3 to 5 μ) which multiplies by budding. It is gram-positive when stained by Gram's method. The tissue form or yeast phase of the organism can be cultivated on Francis' glucose cystine blood agar[2] or brain-heart infusion glucose blood agar at 37° C. (Fig. 1,*B*). After 2 to 3 days' incubation, small, soft, grayish-yellow, bacterial-like colonies appear which contain the typical oval or fusiform budding cells. When planted on Sabouraud's glucose agar and incubated at room temperature the saprophytic moldlike form of the organism develops. After 3 to 5 days' incubation, very small white colonies appear which, after 7 to 10 days' incubation, become moist, membranous, and wrinkled. The color may be cream, brown, or black. The colony is characterized by delicate (2 μ in width), branching, septate hyphae which bear conidia laterally or in groups from the ends of lateral branches (Fig. 1,*C*). The conidia are pyriform, ovoid to spherical, and about 2 to 4 μ by 2 to 6 μ in size. Round, thick-walled conidia may be seen in old cultures. The filamentous form of the organism will revert to the tissue yeastlike form when subcultured on suitable media at 37° C.

*Professor of Microbiology and Associate Professor of Medicine.

The tissue form of the organism has been observed in only a few instances in man although it occurs in abundance in experimentally infected mice and rats.[1] The filamentous phase does not occur in man or experimental animals. The rarity of a recognizable morphologic form in pus, in biopsy, or necropsy specimens emphasizes the importance of cultures for the diagnosis of this mycotic infection.

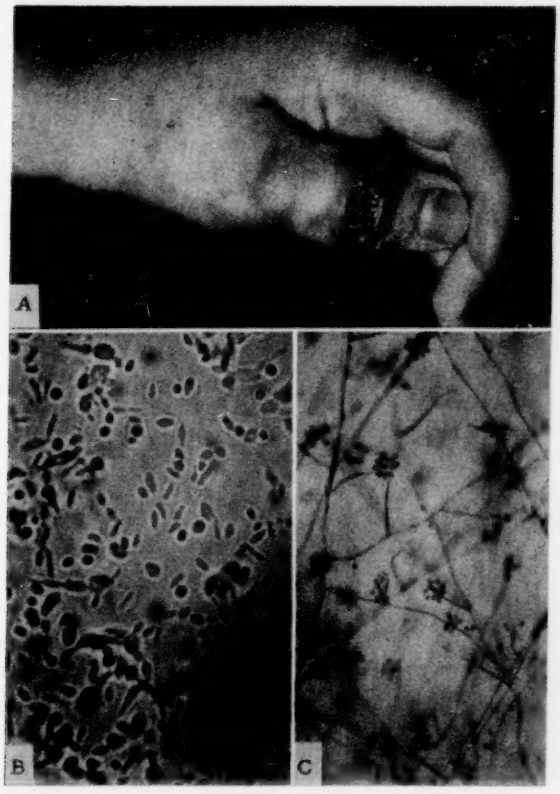


Fig. 1.—*A*, Sporotichotic chancre of the thumb. (From Fogel and Martin, J. Pediat. **17**:193, 1940.) *B*, Yeastlike phase of organism. *C*, Moldlike phase of organism.

The studies of Lurie[4] and Nordén[5] have shown that there is only one antigenic type of *Sporotrichum schenckii*, making possible the use of a stock culture, grown in the tissue phase, for agglutination with the patient's serum or for the preparation of a vaccine for skin tests or desensitization.

There are six different clinical types of the disease; (1) lymphatic, (2) disseminated, (3) epidermal, (4) mucosal, (5) skeletal, and (6) visceral. An occasional patient may have two or more of the clinical forms of the disease simultaneously.[7] The lymphatic form is encountered most frequently and the generalized form is rarest. The primary infection occurs most often on the hands

or fingers. The fungus gains entrance through a gross or microscopic lesion of the skin and grows slowly for 20 to 90 days before the appearance of a hard, spherical, elastic, nontender nodule which is not adherent to the overlying skin. With further growth the nodule becomes attached to the skin which becomes red, then purplish, and finally black and necrotic (Fig. 1,*A*). This sporotrichotic chancre may persist for months or years if it is not recognized and treated. A few days or a week after the appearance of the primary lesion, multiple subcutaneous nodules develop along the course of the lymphatics draining the original site of infection. These secondary lesions may or may not ulcerate. The milder forms of glandular tularemia are difficult to differentiate clinically from the lymphatic form of sporotrichosis.

The primary lymphatic form of the disease can be suspected from the clinical picture and the diagnosis confirmed by culture. The pulmonary, visceral, generalized, skeletal, and other forms of the disease present a real challenge to the diagnostician since there is nothing characteristic about the history or physical findings. Sporotrichosis, along with other mycoses, should be suspected when the patient has multiple polymorphous lesions which fail to respond to ordinary methods of treatment. Agglutinations, skin tests, and complement fixation tests may be done to supplement cultural studies.

The prognosis varies with the clinical form of the disease and its duration. The lymphatic and epidermal types of infection usually respond satisfactorily to iodide therapy. Sporotrichosis of the mucous membranes, whether of the nose, mouth, or larynx, are more difficult to treat because of secondary bacterial infections. The disseminated, visceral, and skeletal forms are rather resistant to therapy.

Potassium iodide is the drug of choice for the treatment of sporotrichosis. It should be given in rapidly increasing doses, beginning with 10 drops of a saturated solution 3 times a day and increasing by 5 drops with each of the 3 daily doses until the patient is receiving 30 to 40 drops 3 times each day. If the clinical response is not satisfactory, sodium iodide, in daily doses of one Gm., may be given intravenously. Open lesions should be compressed with an aqueous iodide-iodine solution containing 2 per cent potassium iodide and 0.2 per cent iodine.

Every effort should be made to avoid secondary infections with bacteria. Collections of pus should be evacuated by aspiration rather than open incision and drainage.

Patience is required for the treatment of the more chronic forms of the disease, since weeks and even many months of iodide therapy may be necessary. If the patient makes only a partial recovery or fails to respond to iodide therapy then certain supplementary methods of treatment should be tried. One of these is the desensitization of the excessively hypersensitive patient. A vaccine is prepared from the yeast-phase culture using a 1:1,000 dilution, in physiologic saline, of the packed yeast cells. The heat-killed vaccine is injected intracutaneously like a tuberculin test and read after 24 to 48 hours. If the test is positive the vaccine is diluted and used for desensitization.

X-ray therapy is used occasionally for isolated lesions. Surgical excision of sinus tracts connected with bone lesions may be necessary to effect adequate

drainage. Propamidine is about as fungicidal for *Sporotrichum schenckii* in vitro as it is for *Blastomyces dermatitidis*.[3] However, hydroxystilbamidine, rather than stilbamidine or Propamidine should be tried in the dose range used for blastomycosis since the latter drugs have more undesirable side effects.

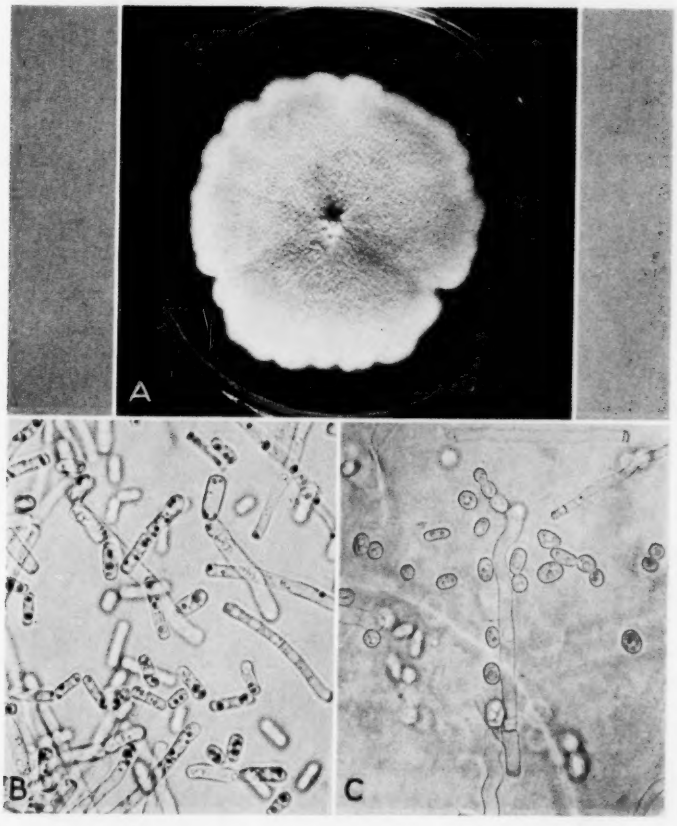


Fig. 2.—*A*, Colony of *Geotrichum candidum* on Sabouraud's glucose agar 10 days at room temperature. *B*, Elongate arthrospores from Sabouraud's glucose agar. (×790; reduced ⅓.) (From Conant, N. F., Am. Rev. Tuberc. **61**:690, 1950.) *C*, Rounded arthrospores from Sabouraud's glucose agar. (×650; reduced ⅓.) (From Smith, D. T. Am. J. Med. **2**:594, 1947.)

GEOTRICHOSIS

Geotrichum candidum can produce disease in the pharynx, conjunctivae, bronchi,[11,12] lungs,[14] skin, and intestinal tract. This fungus, like *Candida albicans*, is a normal inhabitant of the pharynx and intestinal tract.[13] It is frequently a secondary invader[15] and only occasionally a primary pathogen.[9,10]

The fungus grows in the tissues as oblong or rectangular cells, 4 by 8 μ or as large spherical cells 8 μ to 10 μ in diameter (Fig. 2,*C*). The latter may be mistaken for young cells of *Blastomyces dermatitidis*. When grown at 37° C. on Sabouraud's glucose agar both rectangular and spherical cells are produced (Fig.

2,*A*). At room temperature on the same medium, long filaments develop which break up into rectangular arthospores (Fig. 2,*B*). *C. immitis* also produces arthospores on cultivation but the colonies are definitely moldlike and tough, while the colonies of Geotrichum are soft and the arthospores are several times as large as those of *C. immitis*.

Geotrichum candidum produces "thrushlike" lesions when growing on the surface of mucous membranes and may be mistaken for lesions produced by *C. albicans*. In bronchial and pulmonary infections the sputum is gelatinous and mucoid and may be bloodstained. Pure pulmonary infections are rarely encountered, but when they do occur they have a tendency to form thin-walled cavities which may be confused with those produced by *Coccidioides immitis* (Fig. 3).

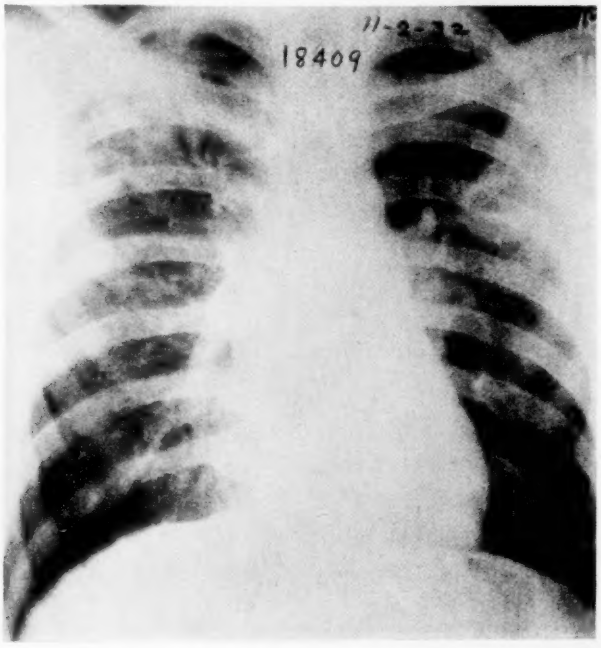


Fig. 3.—Geotrichosis of the lungs. There are two cavities in the right upper lobe and a larger one in the left upper lobe. The lesions disappeared with potassium iodide therapy. (From Smith, D. T. J. Thoracic Surg. 3:241, 1934.)

Oral lesions usually respond to treatment with local applications of gentian violet. We paint the lesions with a 1:10,000 solution of gentian violet in 10 per cent alcohol or have the patient gargle a 1:100,000 solution of the dye. The intestinal form of the disease can be treated with gentian violet in salol-coated capsules in doses of 32 mg. 3 times each day. The bronchial and pulmonary forms usually respond to oral therapy with potassium iodide. If progressive improvement does not follow then hypersensitivity should be suspected and a heat-killed vaccine should be prepared from the patient's own culture. The

methods for preparation of the vaccine and of performing the skin tests have been described in the section on sporotrichosis.

ASPERGILLOSIS

Aspergillus fumigatus, *A. niger*, *A. clavatus*, and *A. versicolor* have been isolated from diseased tissues of man under circumstances which suggest that they were the primary etiologic agent.[19] *A. fumigatus* is a common pathogen of birds,[16] and is the species most frequently associated with diseases in man.

It is difficult to establish the diagnosis of aspergillosis because the fungus is ubiquitous in nature and frequently contaminates cultures in the laboratory. Furthermore, a freshly collected specimen of sputum may become contaminated with Aspergillus spores from the air before it reaches the laboratory. The collection of a specimen under sterile conditions or even removal of a specimen from the bronchi with a bronchoscope does not rule out the possibility of the fungus being a contaminant since the fungus spores, like particles of soot, may have been breathed into the lungs the day before the bronchoscopy.

If hyphal forms of the organism are found in freshly expectorated sputum one can conclude that the fungus is growing in the bronchi or lungs but it may be a secondary invader and not the cause of the disease.

Masses of wide septate hyphae are found growing in the lesions of man. No spore forms are produced unless the lesion extends to a surface where free oxygen is available. This may occur in a bronchus or in a partly excavated but open cavity in the lung.

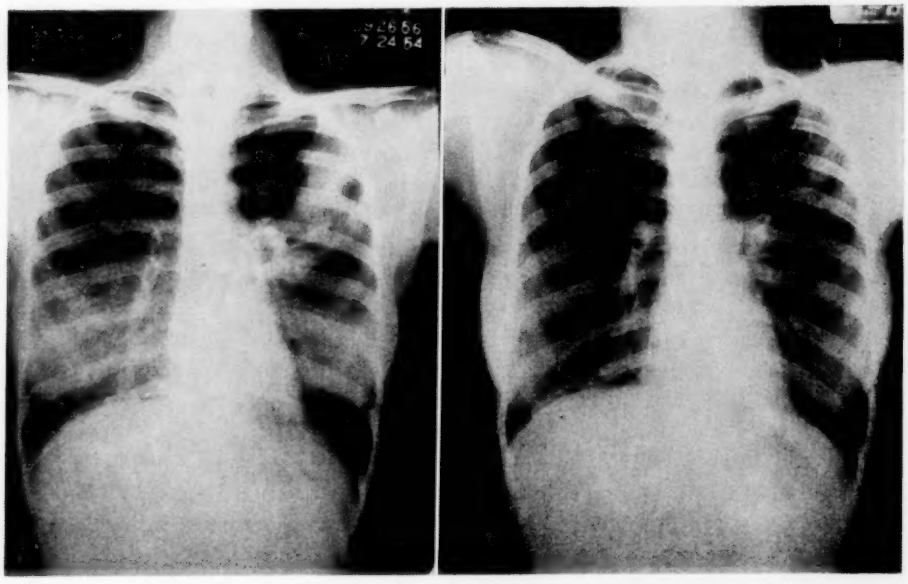
Aspergilli grow readily in 2 to 4 days on Sabouraud's glucose agar producing in the first 24 to 48 hours a basal white feltlike growth consisting of intertwined mycelium of septate hyphae. Certain of the hyphal cells become enlarged and send up a stalk or shoot with a swollen vesicle at its extremity. This structure is known as a conidiophore. Shorter stalks, the phialides, resembling the bristles of a brush, arise from the vesicle. Each of these short stalks bears a chain of conidia or spores which are colored and hence give the characteristic color to the colony.

Aspergilli may produce inflammatory granulomatous lesions in the skin, external ear, nasal sinuses, orbit, eye, bronchi, or lungs,[17,18,20] and occasionally in the bones and meninges.

There has been an apparent increase in the number of proved pulmonary infections since resectional surgery is more frequently employed to establish or eliminate tumors of the lung. Three clinical forms of pulmonary aspergillosis have been recognized. One form presents no characteristic clinical or roentgenographic picture and is usually diagnosed at necropsy or after resection of an infected lobe for diagnosis.[21] The other two clinical forms can be recognized by a combination of clinical and roentgenographic findings.

The first of the recognized forms is an allergic type of migrating pneumonitis,[19] sometimes with an associated increase in the eosinophils of the blood but more often without any increase in these cells. Hyphal filaments can be found in the fresh sputum, and cultures regularly give a heavy growth of the organism.

A heat-killed vaccine prepared from the patient's own culture gives an immediate wheallike reaction resembling those seen in patients with hay fever or asthma. Recurring attacks may occur over a period of years without making the patient seriously ill. The film from a patient with this type of infection is shown in Fig. 4. The second clinically recognized form begins as a single area or as multiple areas of diffuse pulmonary infiltration which over a period of weeks or months become sharply circumscribed, extremely dense circular objects which resemble marbles, golf balls, or even tennis balls. Some excavate, leaving a thin-walled



A. *B.*

Fig. 4.—Allergic type of aspergillosis of lungs. *A*, Shows lesions in left upper and right lower lobes. *B*, Shows almost complete clearing of lesions with minimal residual scarring.

cavity which persists. Those which do not excavate remain as circular bodies for years (Fig. 5). If resected shortly after the definite circular shape has been assumed, microscopic examination reveals a dense fibrous wall surrounding a mass of living hyphae without spore heads. If partly excavated, spore heads may be seen projecting into the air space. After a period of years the unexcavated lesions show a complete disappearance of all fungus elements, leaving a dense, thin, fibrous wall surrounding a homogeneous puttylike material.

The allergic type of migrating pneumonitis can be treated by a process of desensitization employing the patient's own organism as the antigen. Partial desensitization should be effected before iodides are administered since, in some instances, the lesions disappear after desensitization without requiring the use of iodides. There may be an associated bacterial infection in these cases and patients may have the same type of sensitivity to the associated bacteria as to

the Aspergillus. The associated bacteria should be isolated and made into vaccines for skin tests. If the skin tests are positive, they should be added to the Aspergillus vaccine so the patients can be desensitized to fungus and bacteria simultaneously. The lesions which resemble marbles or balls should be resected.

PENICILLIOSIS

Certain species of Penicillium occasionally produce infections in the lungs.[24] The case reported by Aimé and his associates[22] presented the clinical and x-ray picture of a pulmonary abscess. The chronic infection of the urinary tract studied by Gilliam and Vest[23] had the fungus in the bladder and kidneys at necropsy.

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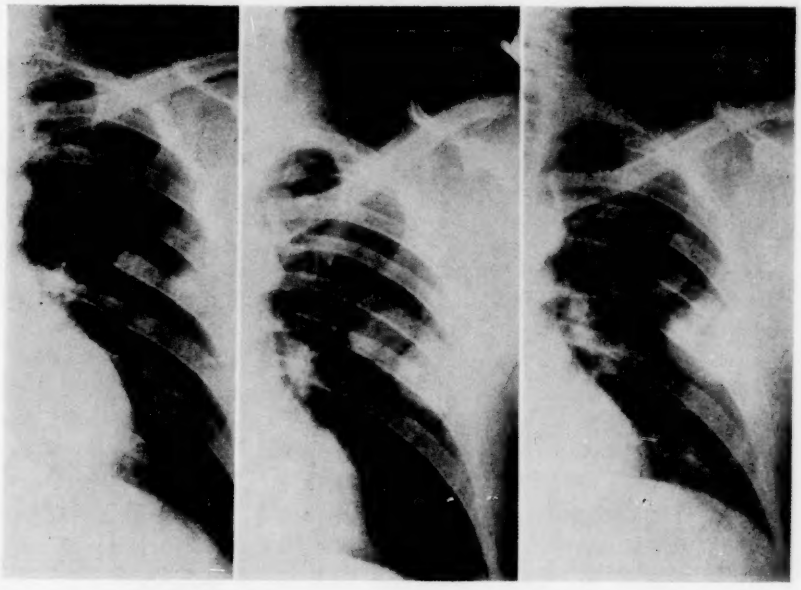

Fig. 5.—Probable case of solid lesions type of aspergillosis. Aspergilli were grown from sputum and from bronchoscopic washings, but not from resected portion of lung. Spores were seen in microscopic sections.

The colony growth of Penicillium resembles that of Aspergillus. The differentiation is made by a microscopic examination of the spore heads. The aerial hyphae do not form a vesicle but branches. The branches produce metulae, which in turn give rise to flask-shaped sterigmata. The conidia occur in unbranched chains from the tips of the sterigmata. The whole structure resembles a brush or "penicillus."

Almost nothing is known about therapy. Presumably this type of infection should be treated by the methods employed for the treatment of aspergillosis.

MUCORMYCOSIS

The order Mucorales includes the genera: Mucor, Absidia, and Rhizopus. All three genera are characterized by the presence of large coenocytic (unseg-

mented) hyphae in the tissues and cannot be differentiated from one another without the isolation of the fungus. The term "mucormycosis" is used for infections caused by all three genera of Mucorales.

These fungi are ubiquitous in nature and were believed to be harmless saprophytes for many years, although Lichtheim[36] demonstrated their pathogenicity for the rabbit in 1884 and, in 1885, Paltauf[38] identified the fungus in a fatal infection of man. The comprehensive review of Gregory, Golden, and Haymaker in 1943[34] stimulated the current interest in this group of fungi. The pathologic reactions both in man and in experimental animals have been studied in detail by Baker and his associates[27-31,33,39,40] and the fungi have been studied by Ajello,[25] Emmons,[41] and their co-workers. Mucormycosis in food-producing animals has been studied by Davis and his associates.[32]

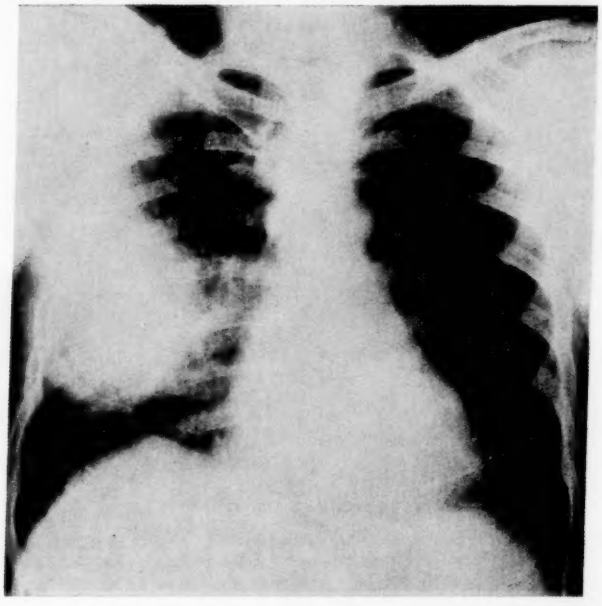

Fig. 6.—Mucormycosis of lungs diagnosed at necropsy. (From Baker, R. D.: Pulmonary Mucormycosis, Am. J. Path. **32**:287, 1956.)

The disease has been recognized only in the United States, Canada, and England, but is probably world-wide in distribution. It occurs most often as a complication of uncontrolled diabetes and leukemia, but may occur after severe burns, or in patients with cirrhosis of the liver and multiple myeloma.[42] Treatment with ACTH, cortisone, antileukemic chemical agents, and antibiotics apparently lowers the resistance of patients to this group of fungi.

The infection may start in the orbit, the paranasal sinuses,[30] bronchi, lungs[27] (Fig. 6), or intestines.[31,37] The organism grows into the wall and lumen of blood vessels from which it metastasizes to the brain (Fig. 7,*A*).

Acute toxic alloxan diabetes in rabbits[26,33] lowers the resistance of the animals to infection with *Rhizopus oryzae* and *Rhizopus arrhizus*. Local invasion of the blood vessel occurs and in many instances metastatic lesions appeared in the brain. Chronic alloxan diabetes does not predispose the animals to infection with these fungi. The administration of cortisone renders rats, mice, and guinea pigs more susceptible to infection with Rhizopus species (Fig. 7,*C*).[40]

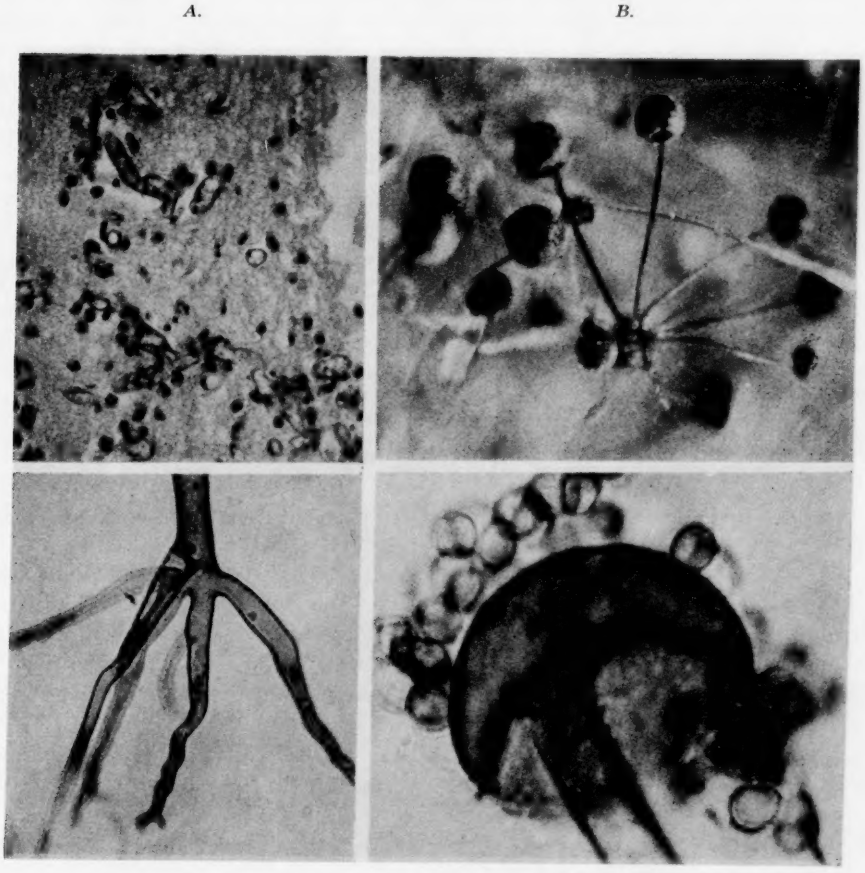

Fig. 7.—Mucormycosis. *A*, Nonseptate hyphae in brain. *B*, Spore heads. *C*, Rhizoid feet characteristic of Rhizopus. *D*, Spore head with isolated spores. (From Bauer, H., Ajello, L., Adams, E., and Hernandez, D. U., Am. J. Med. **18**:822, 1955.)

These fungi grow readily on Sabouraud's glucose agar at room temperature, filling the Petri dish in 5 days with an abundance of floccose aerial mycelium. The nonseptate sporangiospores develop a terminal globose structure which is filled with elliptical spores (Fig. 7,*B* and *D*).

Until recently all of the recognized cases were found at the necropsy table. It is obvious that the diagnosis must be established before the infection has

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spread from the portal of entry to the brain. The first surviving patient was reported by Harris in 1955.[35] This patient was a 12-year-old Negro girl who was admitted to the Duke Hospital with diabetic acidosis, ulceration of the palate, infection of the left ethmoid and maxillary sinuses, ophthalmoplegia with loss of vision of the left eye, and loss of sensation on the left side of the face. The essential part of the treatment was the prompt regulation of the diabetes with insulin. Supplementary treatment consisted of injections of a vaccine prepared from the patient's own strain of Rhizopus, followed by the administration of potassium iodide. The patient has remained well for over two years, although there is residual blindness of the left eye and loss of sensation on the left side of the face. A second case of this type was found in an adult in the Durham Veterans Hospital in 1955. This was an ethmoidal sinus infection in an uncontrolled diabetic. This patient recovered after the diabetes was controlled and is to be reported by Baker, Andrews, and Izlar.[30] In a third case, the primary infection was a pulmonary abscess in an unregulated diabetic. The regulation of the diabetes and the removal of one lung apparently prevented the spread of the infection.

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CHROMOBLASTOMYCOSIS

This mycosis is characterized by the development of warty, or cauliflower-like cutaneous nodules (Fig. 8,*A*) which evolve slowly over a period of years.[43,48] The general health of the patient is not impaired and the organisms do not invade the internal organs. This infection occurs most often in tropical and subtropical countries.[50,51] Only 13 cases have been reported from the United States.[44]

The infection is found most often on the feet and legs but may occur on the hands, face, and other parts of the body.

The organism appears in the tissues as single or clustered, round, thick-walled, dark brown bodies 6 to 12 μ in diameter, which multiply by splitting and not by budding[46] (Fig. 8,*B*). The early verrucous lesions do resemble some forms of dermal blastomycosis. However, the name "chromoblastomycosis" is definably incorrect since the lesions are not pigmented and the organism is not a Blastomyces.

When pus, or bits of tissue containing the tissue form of the fungus are planted on Sabouraud's glucose agar at room temperature, a slowly developing colony appears which is matlike and develops a greenish-brown, olive black, or black pigment after 3 to 4 weeks. Microscopic examination of the fully developed fungus enables the mycologist to differentiate the three species: *Hormodendrum pedrosoi* (Fig. 8,*D*), *Hormodendrum compactum* (Fig. 8,*E*), and *Phialphora verrucosa* (Fig. 8,*C*). All of the species have the same morphologic forms in the tissues,[47] and all three produce the same type of clinical disease.[45,46,52]

Small, well-circumscribed lesions can be cured by surgical excision.[49] The more advanced lesions are resistant to treatment. No cures have been effected, but some improvement has resulted from iodide therapy and from iontophoresis with copper sulfate.[48]

MADUROMYCOSIS

This disease was recognized first in Madras, India, and was described as "madura foot." It usually occurs on the feet but occasionally involves the hands

or some other part of the body.[54,55] The infection is most prevalent in the tropical or subtropical areas where few people wear shoes and the feet come in direct contact with the soil.[53] This mycosis is not infrequent in the southern part of the United States.

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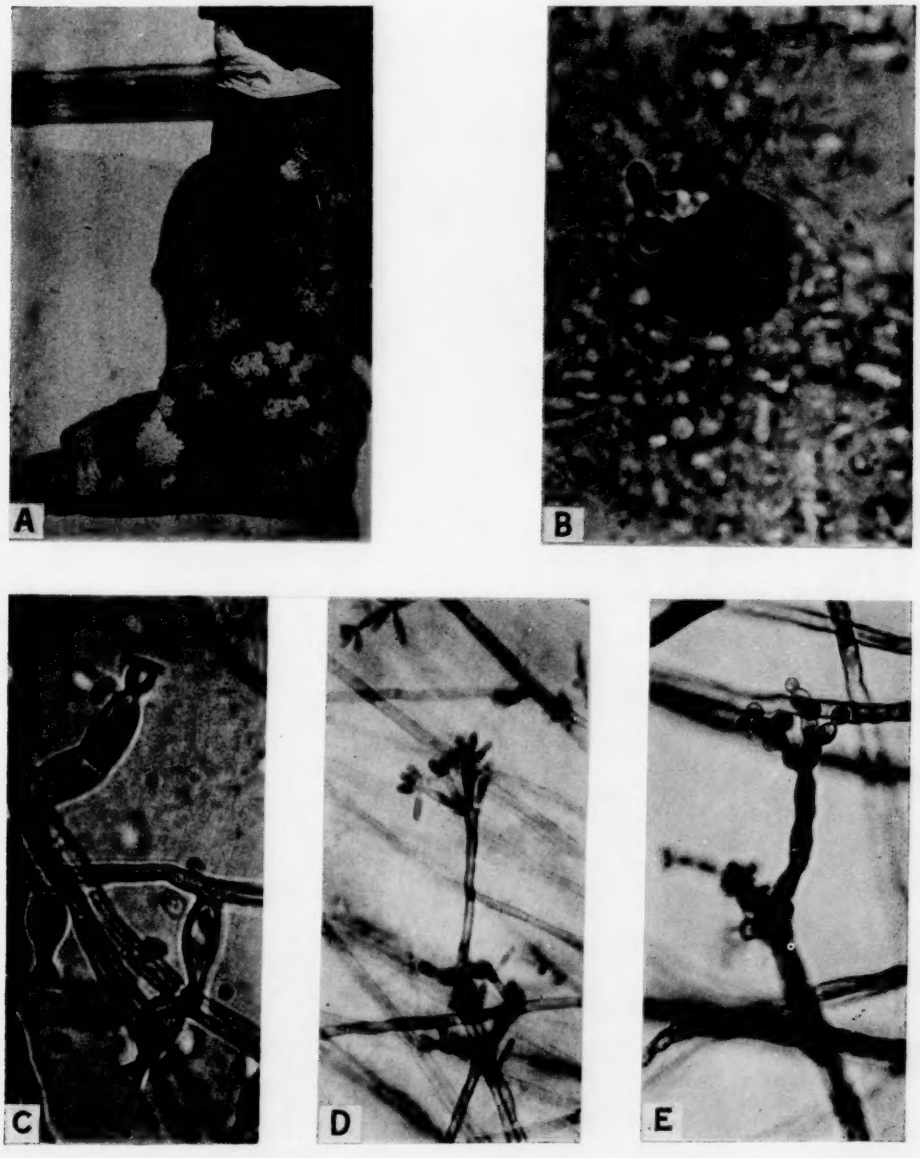

Fig. 8.—Chromoblastomycosis. *A*, Chromoblastomycosis of foot and leg. (From Simson, F. W., and others, J. Path. & Bact. **55**:191, 1943.) *B*, Tissue form of organism. Note dark brown septate bodies in pus. *C*, *Phialophora verrucosa* from culture. *D*, *Hormodendrum pedrosoi* from culture. *E*, *Hormodendrum compactum* from culture. (*B-E*, Courtesy Dr. Norman F. Conant.)

Twenty-six species of fungi[54a,56,57] belonging to twelve genera have been isolated from typical clinical cases of maduromycosis.

The infecting organism gains entrance to the tissues of the foot through a gross or microscopic abrasion. Months or even years are required for the in-

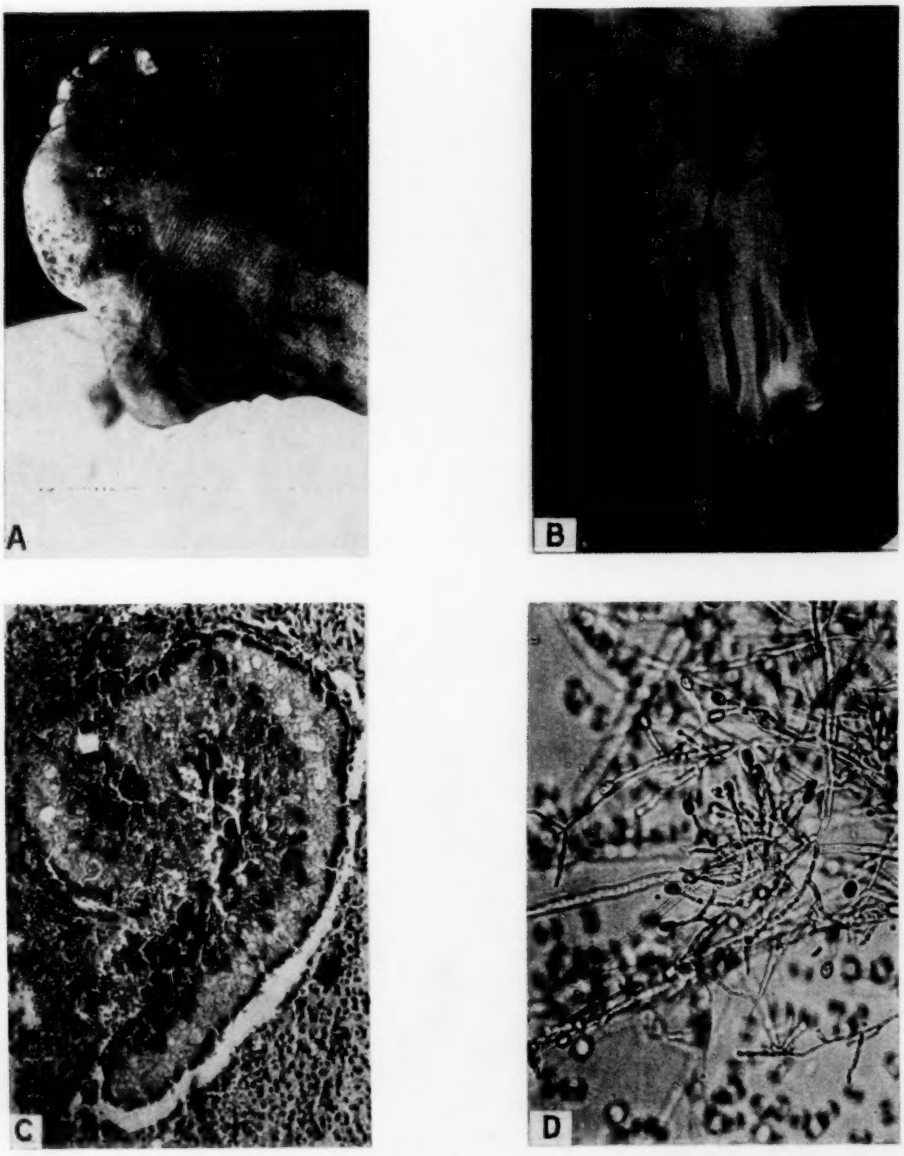

Fig. 9.—Maduromycosis. *A*, Maduromycosis of foot caused by species of Actinomyces. (From Burns, E. J., Moss, S. S., and Brueck, J. W., Am. J. Clin. Path. **15**:35, 1945.) *B*, X-ray of maduromycosis of foot caused by *Monosporium apiospermum*. (From Conant, N. F., and others: Manual of Clinical Mycology, ed. 2., Philadelphia, 1954, W. B. Saunders Company.) *C*, Granule from pus from a case of the moldlike type of maduromycosis. (Courtesy Dr. Norman F. Conant.) *D*, *M. apiospermum* from Sabouraud's glucose agar, room temperature. (From Conant, N. F., and others: Manual of Clinical Mycology.)

vasion of the tissues of the foot as well as the bones (Fig. 9,*B*). The foot appears swollen, discolored (Fig. 9,*A*), and clublike and is honeycombed with sinuses which discharge pus containing white, yellow, orchid, red, or black granules (Fig. 9,*C*). The granules contain the mycelial elements of the specific invading

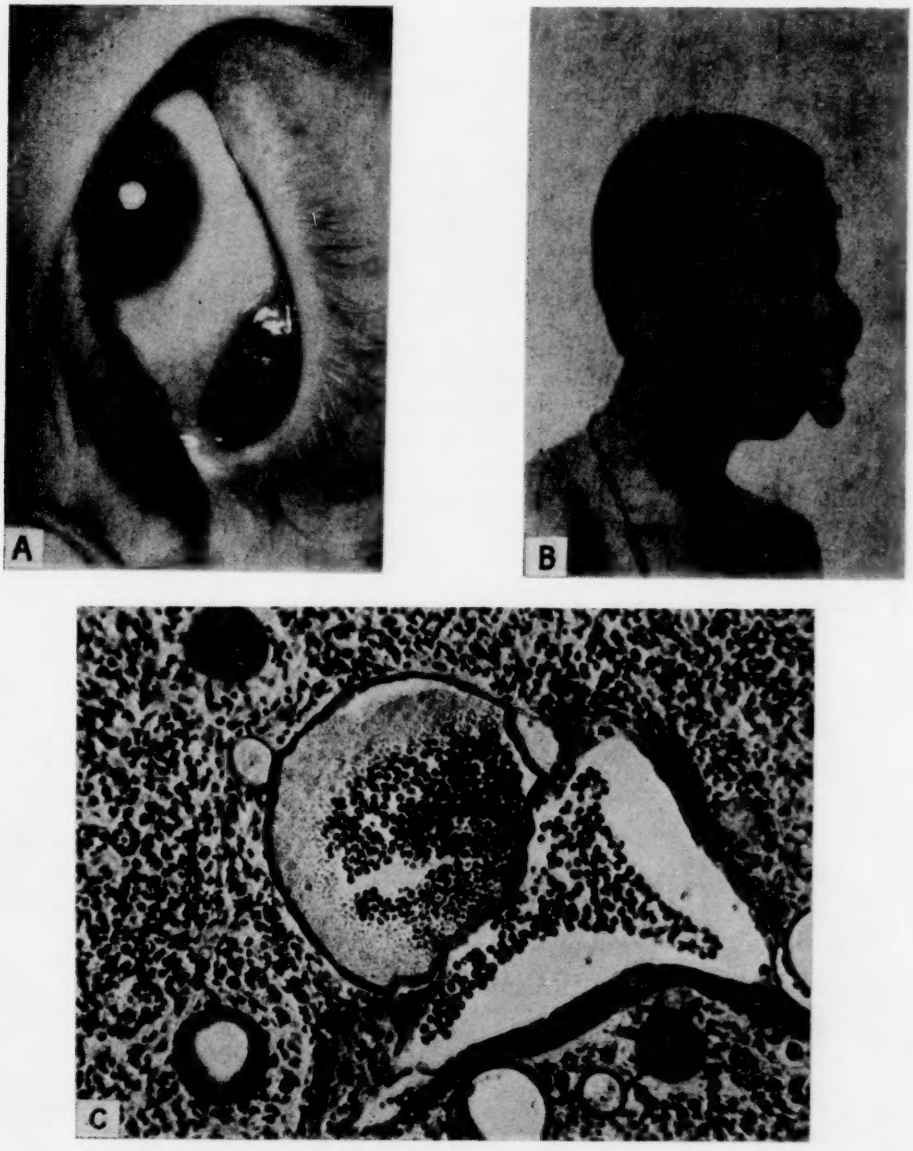

Fig. 10.—Rhinosporidiosis. *A*, Rhinosporidiosis of eye. Note white spots in lesion which contain the spores. (From Arnold, R., and Whilden, J., Am. J. Ophth. **25**:1227, 1942.) *B*, Rhinosporidiosis of nose and face. (From Allen, F.R.W.K., and Dave, M. L., Indian M. Gaz. **71**:376, 1936.) *C*, *Rhinosporidium seeberi* in section of a nasal polyp. Note large sporangium filled with small spores. (From Conant, N. F., and others: Manual of Clinical Mycology, ed. 2, W. B. Saunders Co., 1954.)

fungus. The isolation and identification of the fungus is essential to differentiate the infections caused by (1) *Actinomyces bovis* which responds to penicillin therapy, and (2) Nocardia species which require treatment with sulfonamides, or (3) the infection caused by filamentous fungi for which there is no specific therapy (Fig. 9,*D*). Amputation of the infected foot is the only known treatment for the latter group of infections.

RHINOSPORIDIOSIS

This disease is characterized by the development of friable, sessile, or pedunculated polyps which occur most often on the mucous membranes of the nose (Fig. 10,*B*), but sometimes in the eyes (Fig. 10,*A*), ears, and larynx, and occasionally on the skin or penis and in the vagina.[58,62] This disease is endemic in India and Ceylon and sporadic in other parts of the world including the United States.[60,61] Two patients with this infection were discovered in the Ophthalmic Clinic of the Duke Hospital in a period of less than two years.[59] None was recognized before and none has been seen since.

Microscopic sections of the infected tissues reveal large, 200 to 300 μ, thick-walled sporangia which are filled with hundreds of endospores (Fig.10, *C*). Spores as small as red blood cells may be seen in the tissues, along with immature sporangia in various stages of development.

This fungus has not been cultivated on artificial media. The diagnosis is made by smear or preferably by biopsy.

Isolated lesions can be cured by surgical excision. There is no satisfactory treatment for the far-advanced cases.[58]

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CULTURAL METHODS FOR HUMAN PATHOGENIC FUNGI

LIBERO AJELLO, PH.D.

CHAMBLEE, GA.

From the Communicable Disease Center, U. S. Public Health Service, U. S. Department of Health, Education, and Welfare

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SUCCESS in the isolation of human pathogenic fungi from clinical materials is equally dependent upon the competence of the diagnostic laboratory and the perspicacity of the physician. Since fungi elicit pathologic responses in their hosts similar to those induced by other microorganisms or by neoplasms, the clinician must not fail to consider the mycoses in his differential diagnoses. Many diagnostic errors of perhaps tragic consequence can be avoided by requests that mycologic as well as bacteriologic and histologic examinations be done on appropriate clinical specimens.

The proper collection and types of specimens required by the laboratory for the successful demonstration and isolation of pathogenic fungi have been described in a previous publication and need not be repeated here.[1] It should be emphasized, however, that once an adequate specimen has been collected, it should be delivered to the laboratory without delay to prevent loss of viability and overgrowth by contaminants.

Laboratory procedures employed for the detection and isolation of pathogenic fungi are not difficult. Simple dextrose-peptone agars such as Sabouraud dextrose agar* are the basic media used in the recovery of most fungi from clinical materials. The addition of antibiotics is recommended to suppress competing bacteria and saprophytic molds that would inhibit or prevent the growth of pathogenic fungi. Penicillin and streptomycin (20 and 40 units, respectively, per milliliter of medium) hold down bacterial growth. Due to their thermal instability, they must be added separately to the sterilized and cooled medium just prior to its solidification. However, the thermostable broad-spectrum antibiotic, chloramphenicol,† (0.4 mg. per milliliter) may be substituted for these two antibiotics, and autoclaved in the medium. Along with antibacterial agents, cycloheximide,‡ (0.5 mg. per milliliter), which withstands autoclaving, should be added to the isolation medium to suppress the growth of saprophytic fungi.

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*Available in dehydrated form through several commercial sources.

†Chloromycetin, Parke Davis & Co.

‡Actidione, The Upjohn Co.

Thus through use of a combination* of antibacterial and fungistatic antibiotics, virtually pure cultures of pathogenic fungi are assured even from the most heavily contaminated clinical specimens.[2]

Such media cannot be used for the isolation of Nocardias, *Cryptococcus neoformans*, *Allescheria boydii*, and *Aspergillus fumigatus*, organisms that are sensitive either to the antibacterial antibiotics or cycloheximide.

In contrast to other types of selective isolation media,[3] cycloheximide media do not alter the morphologic characteristics of insensitive fungi. Thus the majority of human pathogenic fungi can be readily isolated as well as identified on the primary isolation media described.

Some species, however, require special methods for their recovery and characterization. In this publication it is proposed to describe specific methods for the culture and identification of only those organisms that present unusual diagnostic difficulties.

Actinomyces bovis.—Although not a fungus, this organism, along with the aerobic pathogenic actinomycetes, traditionally has been relegated to the province of medical mycology rather than bacteriology. Because of its unique physiologic properties, *A. bovis* does not lend itself to study with the usual mycologic techniques. Specific methods must be employed for its isolation and identification.

The choice of isolation procedures for this branched, filamentous, bacterium-like anaerobe is dependent upon the nature of the clinical specimen collected. If derived from a closed sinus tract or other uncontaminated source, *A. bovis* is most successfully isolated in thioglycollate broth† tubes incubated at 37° C. Specimens likely to be contaminated with other bacteria should be serially streaked out on brain-heart infusion agar plates enriched with blood (6 per cent) and incubated at 37° C. in a Brewer anaerobic jar.

In liquid media *A. bovis* tends to develop in the form of whitish discrete aggregates of mycelium. The medium does not become clouded as generally occurs with other bacteria. On agar the colonies rarely exceed 2 mm. in diameter. They are whitish in color, and irregular in form. The organism develops in the form of branched filaments, approximately 1μ in width, that tend to break up into "V" and "Y" shaped fragments. Microaerophilic diptheroids such as *Corynebacterium acnes* have similar colonial and morphologic characteristics, and consequently misidentifications often occur. However, these organisms can be differentiated from *A. bovis* on the basis of biochemical characteristics (Table I).

Candida albicans.—Special media and tests are required to bring out the mycelium and chlamydospores which differentiate *C. albicans* from the true yeasts, cryptococci, and nonpathogenic Candida species, which superficially resemble it on primary isolation media. Sugar-free corn meal agar, a "homemade" variable product, has been the standard medium in many laboratories for inducing the formation of these diagnostic characteristics. Recently several repro-

*Media containing chloromycetin and cycloheximide are available commercially.

†Commercially available from several sources.

ducible media have been developed which greatly facilitate and make more accurate the identification of this fungus.

On "Chlamydospore agar,"* *C. albicans* with notable regularity develops mycelium and chlamydospores.[4] Levine eosin-methylene blue agar* as modified and utilized by Weld[5] is even more efficient. Furthermore, it has the advantage of giving definitive results within 18 to 24 hours in contrast to the 48-hour incubation period generally required with "Chlamydospore agar." With the Weld procedure, clinical materials may be directly inoculated on the eosin-methylene blue plates as the presence of Aureomycin and other inhibitors suppresses the growth of contaminating bacteria.[6]

TABLE I. DIFFERENTIAL BIOCHEMICAL PROPERTIES OF A. BOVIS AND MICROAEROPHILIC DIPTHEROIDS

ORGANISM	BIOCHEMICAL REACTIONS				
	CATALASE PRODUCTION	LIQUEFACTION OF GELATIN	PEPTONIZATION OF MILK	INDOLE FORMATION	NITRATE ASSIMILATION
A. bovis	−	−	−	−	−
Microaerophilic Diptheroids	+	+	+	+	+

Cryptococcus neoformans.—Cryptococci and other unicellular imperfect yeasts are frequently encountered in diagnostic work. Although most of these organisms can, in general, be distinguished from *C. neoformans*, some present taxonomic difficulties. This is especially true of the nonpathogenic species of cryptococcus, *C. laurentii*, *C. terreus*, *C. albidus*, *C. luteolus*, and *C. diffluens*. All of these species may produce large capsules and thus appear to be morphologically similar to *C. neoformans*. While pathogenicity tests and growth at 37° C. serve to separate *C. neoformans* from other members of the genus, inconclusive reactions frequently occur to cast doubt on the identity of this organism. The use of assimilation tests as formulated by Lodder and Kreger-Van Rij[7] have been found indispensable in this laboratory in the identification of *C. neoformans*. Their use has been greatly simplified by the availability of the necessary test media in dehydrated form.†

The urease test recently described by Seeliger[8] has been utilized for more than a year in our laboratory and found to be a most useful procedure for yeasts and yeastlike organisms. All nonpigmented, urease-positive isolates are considered to be Cryptococci and are then subjected to assimilation and other tests for further classification.

Coccidioides immitis.—As a result of widespread travel to and from the endemic areas, cases of coccidioidomycosis are apt to appear in all parts of the

*Commercially available in dehydrated form.
†Difco: "Yeast Nitrogen Base"; "Yeast Carbon Base."

United States. Thus diagnostic laboratories in any geographic location may be called upon to isolate and identify *C. immitis*.

The hazards of handling this highly infectious mold may be minimized by utilizing indirect procedures for its detection in clinical materials. All types of specimens may be injected directly into animals without the necessity of making cultures. Sputum, gastric washings, and other materials are treated with 1,000 units of penicillin and 1,000 units of streptomycin per milliliter, respectively. With intermittent shaking, the specimen is held for 1 hour at 37° C. and then centrifuged at 3,200 r.p.m. for 20 minutes. After removal of the supernatant, the sediment is suspended in 2 to 4 ml. of sterile saline and 1 ml. is injected intraperitoneally into each of 4 mice.

If the mice do not die within 4 weeks, they are sacrificed and smears are made from any lesions that may be present. These are examined for the presence of thick-walled sporangia (20 to 200μ in diameter) that are filled with endospores.

Guinea pigs may be infected intratesticularly with 0.1 ml. of the antibiotic-treated clinical specimen. Within 2 weeks a marked orchitis will develop if *C. immitis* was present in the inoculum. Fluid may then be withdrawn from the testes with a syringe and examined microscopically. If an orchitis does not develop, the animal is held for 4 weeks, sacrificed, and testicular material is examined for sporangia.*

Histoplasma capsulatum.—Diagnosis of the highly prevalent benign form of histoplasmosis is generally based on immunologic and roentgenologic findings. The laboratory isolation of *H. capsulatum* from patients with this form of the disease is rarely achieved. Cultural failures probably are due, in part, to the scarcity of the fungus in the patient's sputum and blood, as well as to the sensitivity of *H. capsulatum* to natural inhibitory agents found in most isolation media.[8a]

The liver-spleen glucose blood agar recently developed by Littman[9] may yield a higher percentage of positive cultures than other media. Controlled laboratory experiments with seeded sputum indicated that this medium permitted the growth of small numbers of the tissue phase cells of *H. capsulatum*. The performance of this medium with routine specimens awaits evaluation.

A proved and efficient procedure for the recovery of *H. capsulatum* from sputum has been described by Moffit, Morgan, and Cameron.[10] This technique takes advantage of the remarkable susceptibility of mice to infection with as few as a single spore of *H. capsulatum*[11] and the ability of the fungus to multiply within the internal environment of the mouse.

In this procedure, sputum is pretreated with antibiotics, and, after homogenization and centrifugation, the sediment is inoculated intraperitoneally into mice. After 4 weeks, portions of their livers and spleens are cultured.

Dermatophytes.—Until recently, a good deal of confusion has existed regarding the identification and classification of members of this group of fungi. Failure to recognize the extraordinary range of morphologic and physiologic variations that can be assumed by dermatophytes led to the creation of a host of "species"

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*Based on procedures developed by the State of California Department of Public Health.

whose true identities are only now coming to light. Monospore cultural studies have demonstrated that many of them belong to a single species. In addition, investigations into the physiology of dermatophytes have shown that many have nutritional patterns that are of diagnostic value (Table II).

The procedures for determining these have been described in several publications by Georg and Swartz.[12-15] Naturally all such tests must be correlated with the gross and microscopic morphology of the isolate before it can be identified.

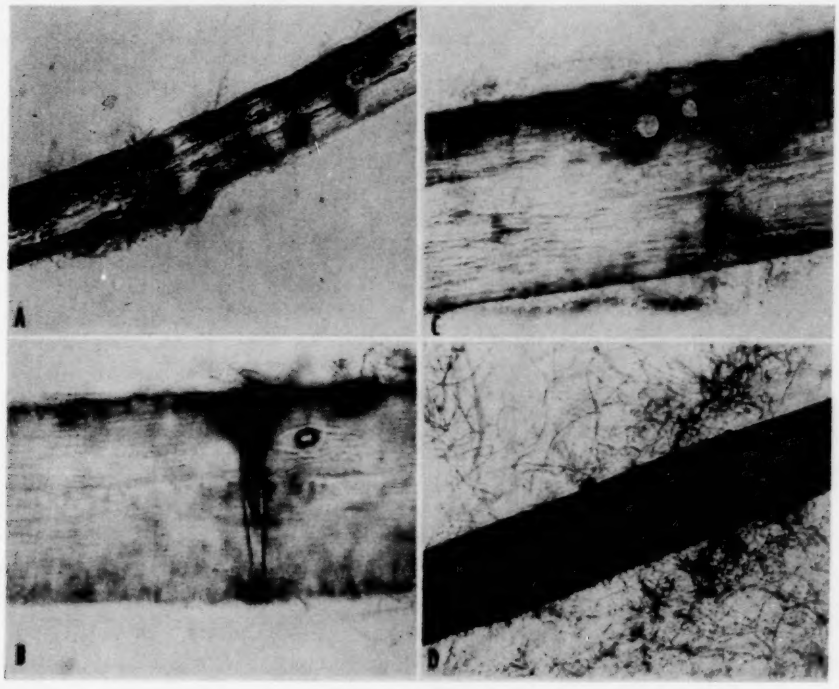

Fig. 1.—*A*, Hair filament with multiple perforations caused by *T. mentagrophytes*. (×220; reduced ½.) *B*, Median optical view of a perforation extending through the full width of a hair filament. (×760; reduced ⅓.) *C*. Surface view of perforations. (×760; reduced ⅓.) *D*, Failure of *T. rubrum* to perforate hair after 3 weeks of growth. (×220; reduced ½.)

A different type of test has been developed for differentiating from each other atypical cultures of *T. mentagrophytes* and *T. rubrum*,[16] two autotrophic organisms. The test is based upon the fact that *T. mentagrophytes* perforates detached hair filaments and *T. rubrum* does not. A Petri dish containing sterilized strands of human hair suspended in water containing yeast-extract* is inoculated with small portions of the unknown organism and incubated at 25° C. Within one or two weeks the hairs are covered with mycelium. Hairs mounted in lactophenol cotton blue and gently heated are examined microscopically for the presence or absence of perforations (Fig. 1).

*One-tenth milliliter of 10 per cent yeast extract per 25 ml. distilled water.

TABLE II. NUTRITIONAL PATTERNS FOR SELECTED DERMATOPHYTES

DERMATOPHYTES	AUTOTROPHIC	REQUIRE THIAMINE	REQUIRE INOSITOL	REQUIRE HISTIDINE	REQUIRE NICOTINIC ACID
T. concentricum†	+	+*			
T. equinum					+
T. ferrugineum	+				
T. gallinae	+				
T. megninii				+	
T. mentagrophytes	+				
T. rubrum	+				
T. schoenleinii	+				
T. tonsurans		+*			
T. verrucosum‡		+ +	+		
T. violaceum		+*			

*Stimulated by thiamine. Requirement is not absolute.

†Fifty per cent of isolates autotrophic; 50 per cent require thiamine.

‡Two patterns found in this species. Eighty-four per cent of isolates require thiamine and inositol; 16 per cent require thiamine only.

It is obvious that medical mycology is anything but a static science. In the diagnostic field, new and improved methods are constantly appearing and are becoming a part of established laboratory practice. Morphologic characteristics are no longer deemed adequate for the identification of many of the human pathogenic fungi and have been supplemented by more reliable physiologic criteria.

In this paper a selection of worth-while precedures, both old and new, has been described which make diagnostic laboratory work more precise. It is to be expected that in the future new knowledge concerning human pathogenic fungi will be applied to the development and utilization of more specific tests for these microorganisms.

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THE DIAGNOSIS OF FUNGUS DISEASES BY BIOPSY

ROGER DENIO BAKER, M.D.*

DURHAM, N. C.

From the Department of Pathology, Duke University School of Medicine and from the Veterans Administration Hospital, Durham, N. C.

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THE accurate diagnosis of many of the deep fungus infections is possible by the histopathologic examination of tissue removed by biopsy.[1,2] This method is valuable in diagnostic studies of the superficial mycoses and should be used more frequently.

The examination of tissue removed by biopsy permits the observation of the fungus in relationship to the characteristic inflammatory response. This reaction is indication that the fungus is not simply a contaminant but the agent responsible for the lesion, and supplements the demonstration of the fungus in direct preparation or culture.

Although tissue is usually obtained by surgical excision, the use of needle biopsy is valuable to obtain bone marrow and liver. The resection of pulmonary lesions has greatly increased the opportunity of recognizing fungus infections of the lungs.

This paper discusses the use of tissue embedded in paraffin and sectioned by conventional methods. It is unfortunate that often only the histopathologic method may be employed, because all the tissue has been placed immediately in fixative at surgery. A portion should be cultured or preserved unfixed, should the tissue study indicate infection rather than tumor. How many regrets this procedure would save! Moreover, the gross specimen should be preserved until microscopic studies are complete, as additional blocks from other sites may be needed.

Staining by hematoxylin and eosin is adequate for the diagnosis of most of the deep fungus infections. Special stains are applied to additional sections cut from the paraffin block. If these are not available, the hematoxylin and eosin staining of a section may be easily replaced by one of the special stains. The cover slip is removed by placing the slice in warm xylol in the paraffin oven for several hours. After rehydration the special stain is applied. Several stains can be used, one after the other.

The most helpful special stains[3,4] are the Gram[1] (Figs. 1, 2, 5, and 7), the Gridley[5] (Figs. 2 and 3), the periodic acid-Schiff, abbreviated to PAS[6] (Figs.

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*Professor of Pathology, Duke University School of Medicine; Chief, Laboratory Service, Veterans Administration Hospital. Address: Veterans Administration Hospital, Durham, N. C.

1, 2, 4, and 7), the mucicarmine[7] (Fig. 4), and the silver[8] (Figs. 3 and 6). Each has its particular virtue. The Gram stain is indispensable in demonstrating the filaments of Actinomyces and Nocardia; the Gridley stain is ideal to scan microscopic fields for fungi, since the background is neutral; the PAS stain demonstrates sharply nearly all fungi except Actinomyces and Nocardia; the mucicarmine stain is specific for Cryptococcus; and the silver impregnations give sharp delineation of organisms.

Diagnostically the two most useful stains for fungi are the Gram and the Gridley. The acid-fast stain is applied routinely because tuberculosis is often a differential possibility.

Keratin may absorb dyes diffusely and obscure the staining of fungi in the stratum corneum of the skin. Staining with methylene blue (Fig. 8) or by the method of Giemsa obviates this difficulty.

In human lesions the fungus usually fails to present the variety of forms seen in cultures. Rounded forms in tissue characterize some of the mycoses, while filamentous (hyphal) forms characterize others, as is evident in the discussion which follows.

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ACTINOMYCOSIS

The Fungus.—Actinomyces bovis occurs as gram-positive, branching filaments 1 μ in thickness (Fig. 1). These are usually collected in mycelial masses called sulfur granules or grains. In hematoxylin and eosin preparations the granules have a diffuse eosin stain, at times with peripheral "clubs" at the terminations of the hyphae. In the PAS preparation (Fig. 1) the granule also stains diffusely. In order to bring out the filaments of the granule the Gram stain *must* be used (Fig. 1).

The Inflammatory Response.—The sulfur granule is found within a minute abscess (Fig. 1), which is, in turn, surrounded by scar tissue containing lymphocytes and plasma cells. At the periphery of the abscess macrophages may be numerous, sometimes in the form of lipophages. Giant cells occur rarely. The disease is a chronic suppurative one.

Clinical Correlation.—The lesions are most frequent in the jaw, oral cavity, tongue, abdominal organs, and lungs. Actinomycotic appendicitis and periappendiceal abscess may be recognized first by histologic study of tissue removed at appendectomy or drainage of abscess. Direct examination of pus for sulfur granules should precede attempts to culture the anaerobic organism. The reason that the lesions are found in the locations indicated is that the infection is endogenous and usually develops via the digestive tract, or, less commonly, the respiratory tract. Hematogenous spread from the region of the appendix to the liver is common, with extension of the hepatic abscess to the subphrenic region, pleura, and lung; generalized systemic infections are uncommon.

NOCARDIOSIS

The Fungus.—Nocardia asteroides is identical to the fungus of actinomycosis, occurring in gram-positive, branching filaments 1 mm. in thickness (Fig.

2). The difference is that these filaments do not form grains or granules, except in mycetomas of the extremities. The filaments are not recognized in hematoxylin and eosin, PAS, or Gridley stains, but are readily demonstrable in Gram stain.

The Inflammatory Response.—The organisms provoke a polymorphonuclear response, with abscess formation, but in some lesions fibrin and caseous necrosis are present. Segments of the hyphae may be engulfed by macrophages. Fibrosis is common in mycetoma, where there are granules, and giant cell response is not infrequent in nocardial mycetoma, the giant cells lying against the granules.

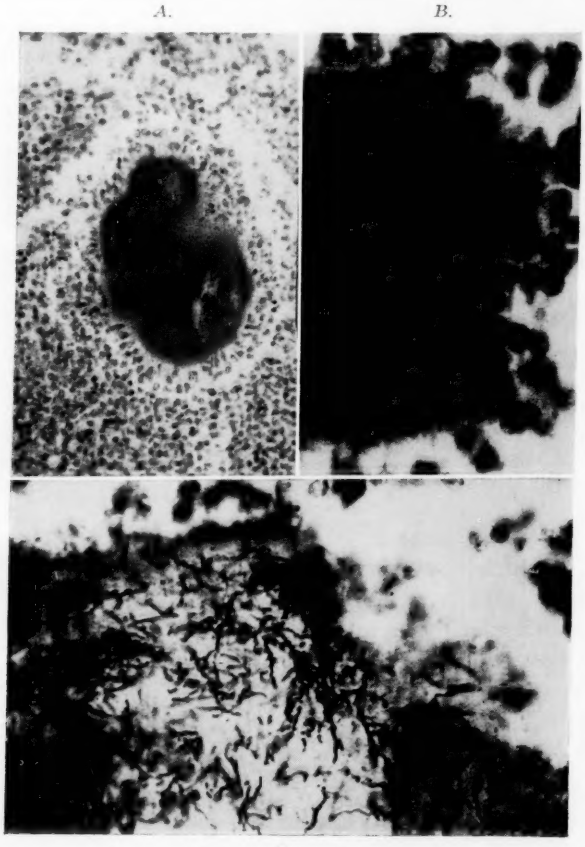

Fig. 1.—*A*, Actinomycosis. Sulfur granule within an abscess. (Hematoxylin and eosin.) *B*, Actinomycosis. Sulfur granule. Note the failure of the PAS stain to demonstrate individual hyphae in the granule. (Periodic acid-Schiff stain.) *C*, Actinomycosis. Sulfur granule. Sharp delineation of the gram-positive, branching filaments composing the granule. (MacCallum bacterial stain.)

Clinical Correlation.—As nocardiosis is an exogenous infection, it develops as a primary pulmonary disease by inhalation or from a puncture wound of an extremity. The pulmonary disease may be extensive and even fatal, with massive, necrotizing pneumonia.[9] Smaller foci may be encountered in pulmonary tissue removed surgically, and here the Gram stain is mandatory.[10] The disease

spreads from the lungs, on occasion, to produce a pyemia or a solitary abscess of the brain or kidney. Aerobic culture and gram-staining should be undertaken.

NORTH AMERICAN BLASTOMYCOSIS

The Fungus.—Blastomyces dermatitidis is a thick-walled sphere, 8 to 15 μ in diameter, with single budding. The organisms are readily seen in hematoxylin, PAS, Gridley, and silver strains (Fig. 2). Gram-staining is capricious, only a portion of the organisms giving the gram-positive reaction. The central portion of the organism is separated by a slight space from the capsule, an important point of difference from developing forms in coccidioidomycosis.

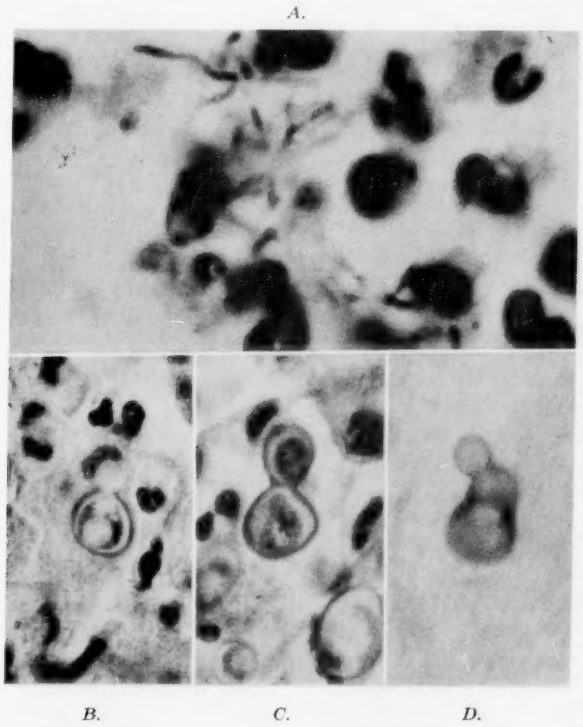

Fig. 2.—*A*, Nocardiosis. Pulmonary lesion. Gram-positive, branching filaments, like those of actinomycosis, but not occurring in granules. (MacCallum bacterial stain.) *B*, North American blastomycosis. Note the space between the thick capsule and the central material. (Hematoxylin and eosin.) *C*, North American blastomycosis. Budding blastomycete in center. (Periodic acid-Schiff stain.) *D*, North American blastomycosis. Budding blastomycete. The neutral background of this stain is helpful in scanning slides for the presence of organisms. (Gridley stain.)

The Inflammatory Response.—The disease is a chronic, suppurative one, with other forms of reaction in varying degree. In acute systemic lesions the organisms may grow massively, interspersed with a few polymorphonuclear neutrophils, giving a yellow, caseous appearance grossly. In older lesions, giant cells contain organisms, and caseous tubercles may be found. Miliary dermal and epidermal abscesses characterize cutaneous blastomycosis.

Clinical Correlation.—Pulmonary lesions alone may be present in some cases, and a nodule of the lung may be subjected to biopsy. The diagnosis can be made by frozen section or by examination of wet mounts of material scraped from the cut surface of the lesion. Cultures should be obtained. Pleural and laryngeal extension from blastomycosis of the lung is common. Blastomycotic tissue from the larynx may resemble squamous-cell carcinoma because of the epithelial hyperplasia caused by the chronic inflammatory response to the fungus. The consequences of an erroneous diagnosis of laryngeal carcinoma are so great, i.e., laryngectomy, that the possibility of blastomycosis or other chronic inflammation must always be considered. If there is any doubt, Gridley and PAS stains should be used.

In systemic blastomycosis, biopsy of lesions of bone, skin, lymph node, and brain may reveal the diagnosis.

Usually cutaneous blastomycosis contains so few organisms that they are difficult to find. The miliary abscesses and the epidermal hyperplasia suggest the diagnosis. The Gridley stain is of most value in searching for the fungus.

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SOUTH AMERICAN BLASTOMYCOSIS

The Fungus.—The single cells of *Blastomyces brasiliensis*, from 10 to 30 μ in diameter, are like those of *Blastomyces dermatitidis*. The distinguishing feature in South American blastomycosis is the presence of multiple buds, which may be large or small. Special stains are of great value in demonstrating multiple budding (Fig. 3).

The Inflammatory Response.—This is similar to that of North American blastomycosis and coccidioidomycosis.

Clinical Correlation.—Biopsy material from lymph nodes or oral-cutaneous lesions is frequently encountered.

COCCIDIOIDOMYCOSIS

The Fungus.—A developing form of *Coccidioides immitis*, frequently seen in sections stained with hematoxylin and eosin, has a thick wall (Fig. 3) and hematoxylin-stained internal material continuous with the capsule. The lack of a space between the capsule and the internal material differentiates this form from the tissue forms of North American blastomycosis. Sporangia, 20 to 60 μ in diameter, containing endospores 2 to 5 μ in diameter, may be found, as well as free endospores. In some lesions only the empty shells of sporangia remain. The Gridley stain is best for scanning lesions in search of organisms, while the PAS stain demonstrates the cells well. Hyphae with arthrospores may be found in pulmonary lesions.

The Inflammatory Response.—This response is similar to that of blastomycosis. Suppuration is common, and neutrophilic reaction to the released endospores is often prominent. Developing forms are encountered in giant cells, or in fibrotic tubercles.

Clinical Correlation.—Organisms may be found in nodules or cavitary lesions removed surgically from the lungs, even when the lesions are calcified.[12] Biopsy material of this sort may be treated in a variety of diagnostic manners. The

cut surface of the lesion can be scraped with a knife and the material so obtained examined in unstained moist preparations. Material can be inoculated into the peritoneal cavities of mice with sacrifice of the mice after a week. Tissue sections of the lesions of the peritoneal surfaces of the injected mice show the organisms in various developmental forms.[13]

Lesions from other regions may be similarly examined, such as those from the skin, the lymph nodes, or the meninges.

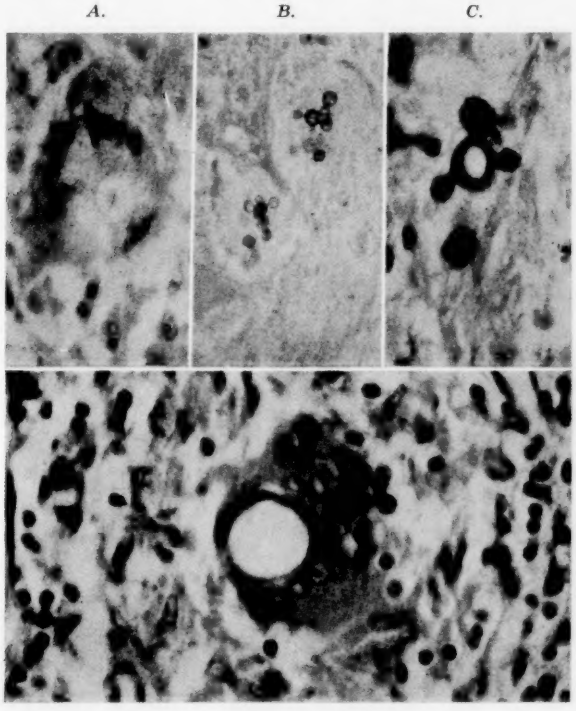

Fig. 3.—*A*, South American blastomycosis. Budding organism in giant cell. (Hematoxylin and eosin.) *B*, South American blastomycosis. Multiple budding. (Gridley stain.) *C*, South American blastomycosis. Multiple budding. Gomori's methenamine-silver nitrate technique. *D*, Coccidioidomycosis. Thick-walled fungus cell in giant cell. Histopathologic diagnosis is possible from this photograph because of the thick wall with a suggestion of inner material beneath the capsule. *Blastomyces dermatitidis* would show a central structure separated from the capsule, as in Fig. 2. Tissue from this case was inoculated intraperitoneally into mice, and all stages of *Coccidioides immitis* demonstrated in sections of the lesions of the peritoneum of the mice. (Hematoxylin and eosin.)

Culture of the fungus in the fluffy, mycelial form may produce infection in the laboratory worker; histopathologic diagnosis or inoculation into mice in such instances may be reasonable substitutes for culture.

HISTOPLASMOSIS

The Fungus.—Histoplasma capsulatum in lesions measures from 1 to 3 μ in diameter (Fig. 4). Hematoxylin-stained material occurs as a mass surrounded

by a capsule. The minute organisms are seen within the cytoplasm of macrophages. The PAS technique causes the capsules to stand out sharply. The Gridley stain is of value, and Gram stains are sometimes excellent.

The Inflammatory Response.—The organism is usually within macrophages. When there are large masses of the fungus-containing macrophages the central portion may become necrotic.[14] Organisms may be demonstrated in old and calcified lesions in histologic section.[15]

Clinical Correlation.—Tissue may come from lesions of the lung, the lymph nodes, the skin, or the mucous membrane of the oral cavity.[16] In systemic cases the bone marrow provides diagnostic tissue.

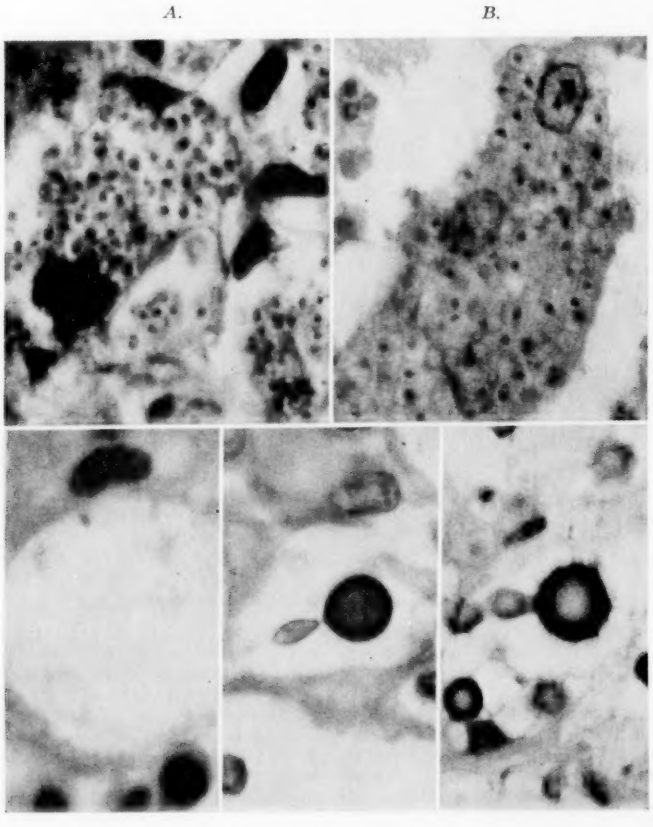

Fig. 4.—*A*, Histoplasmosis. Organisms within macrophages. (Hematoxylin and eosin.) *B*, Histoplasmosis. Organisms within a large macrophage. The capsules of the organisms stain more sharply than in the hematoxylin and eosin preparation. (Periodic acid-Schiff stain.) *C*, Cryptococcosis. Shadowy outlines of organisms surrounded by gelatinous material, in the cytoplasm of a giant cell. Organisms may fail to stain in routine hematoxylin and eosin preparations. Moreover, the organisms have been partially digested in the giant cell and may stain less well on this account. (Hematoxylin and eosin.) *D*, Cryptococcosis. Brilliantly stained budding organism surrounded by gelatinous capsular material. (Periodic acid-Schiff stain.) *E*, Cryptococcosis. Brilliantly stained budding organism surrounded by gelatinous capsular material. This method is specific for *C. neoformans*. (Mucicarmine stain.)

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CRYPTOCOCCOSIS

The Fungus.—Cryptococcus neoformans is a round, budding organism 5 to 20 μ in diameter surrounded by a wide, gelatinous capsule (Fig. 4). The organism may fail to stain in hematoxylin and eosin preparations. The PAS, the Gridley, and silver stains are all effective. The gelatinous capsule usually remains unstained. Mucicarmine preparations are specific for the fungus. When the organisms are within the cytoplasm of giant cells they often stain poorly and are much reduced in size, resembling vacuoles. Small forms are easily confused with those of histoplasmosis.

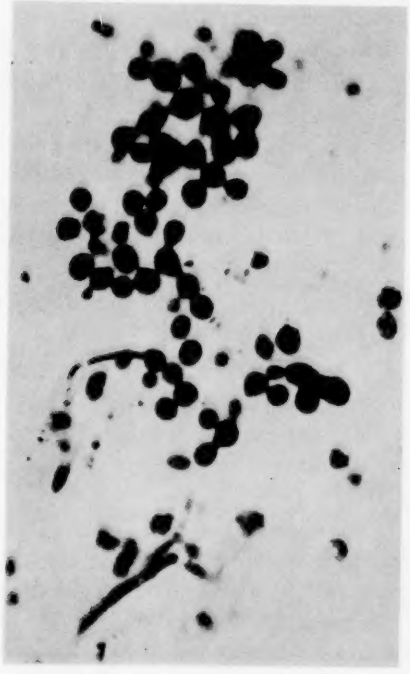


Fig. 5.—Candidiasis. Lesion of esophagus in which rounded fungus cells predominate over hyphae. In other cases the hyphae may predominate. (MacCallum bacterial stain.)

The Inflammatory Response.—The organisms may grow luxuriantly with prominent capsules, causing little inflammation. Often, however, the reaction is chronic, with giant cells, lymphocytes, a few polymorphonuclear cells, and fibrosis.[17] Suppuration is rare and calcification absent.

Clinical Correlation.—Biopsy and excised material comes from the lung or brain, rarely from skin or bone. Pulmonary nodules resected operatively have varied from 3.5 to 7.5 cm. in diameter, have occurred in various lobes of the lungs, and have been solid granulomas except for central necrosis and for cavities in larger lesions.[18,19] In the brain, lesions may simulate primary neoplasms, though the meninges are more commonly affected. When the organisms have

generous capsular material the gross specimen has a gelatinous or jellylike character, similar to that of mucoid carcinoma. Skin lesions occur uncommonly, and contain demonstrable organisms.

CANDIDIASIS

The Fungus.—*Candida albicans* occurs in tissues either in filamentous or in yeast form, or in a combination of the two (Fig. 5). The oval, budding cells measure 2 to 4 μ across. Hyphae may bear spores at points of constriction. Hematoxylin and eosin, Gram, PAS, and Gridley stains are effective. The fungus is gram-positive, but often fails to stain completely.

The Inflammatory Reaction.—Often the lesion, in the form of a white patch, consists largely of the organism in mycelial growth, with loss of epithelium and moderate ordinary chronic inflammation. In deep lesions, abscesses and giant cells form.

Clinical Correlation.—Lesions subject to biopsy are seen most frequently in the oral cavity and on the skin. Growth along the esophagus, in gastric ulcers, and in the cavity of the uterus has been noted. Cutaneous abscesses, endocarditis with septicemia, and meningitis have been reported. Bronchopulmonary lesions are not usually subjected to biopsy.

ASPERGILLOSIS

The Fungus.—In deep lesions the fungus is seen in segmented hyphal form with Y-shaped branchings. The hyphae are usually less than 5 μ thick. Brown spore heads with innumerable brownish spores 2 to 3 μ in diameter may be found in pulmonary cavities, in air sinuses, or in the external ear canal. The PAS and Gridley stains are effective.

The Inflammatory Response.—Abscess formation characterizes disseminated pyemias. Pulmonary lesions may exhibit little inflammatory response, or chronic inflammation and necrosis may be noted. The fungus may invade blood vessels as do the organisms of mucormycosis.

Clinical Correlation.—Aspergillosis often develops secondarily in a predisposing disease. A tuberculous cavity may develop aspergillosis secondarily.

MUCORMYCOSIS

The Fungus.—In tissue, Rhizopus, Mucor, and Absidia form broad, branching, nonseptate hyphae up to 20 μ broad (Fig. 6). The organisms are often inconspicuous but can be demonstrated in hematoxylin and eosin preparations.[20,21] Silver impregnations stain the organisms well. The poor delineation of the organism with other stains is due to the delicacy of the peripheral membrane and the paucity of stainable internal material. In most cases, only hyphae occur, but sporangia have been reported in a case involving an ethmoid air sinus.[21]

The Inflammatory Response.—The inflammatory response is acute, with polymorphonuclears. The organism grows into arteries and veins, causing thromboses and infarcts.

Clinical Correlations.—Mucormycosis is an acute mycosis as a rule. Many cases of cerebral and pulmonary mucormycosis develop in persons who have a predisposing disease or condition, especially diabetes mellitus or leukemia.

Diagnosis by biopsy has been made from tissue from the roof of the mouth,[22] from an air sinus, and from the lung. Rhizopus has been the fungus most frequently cultured.

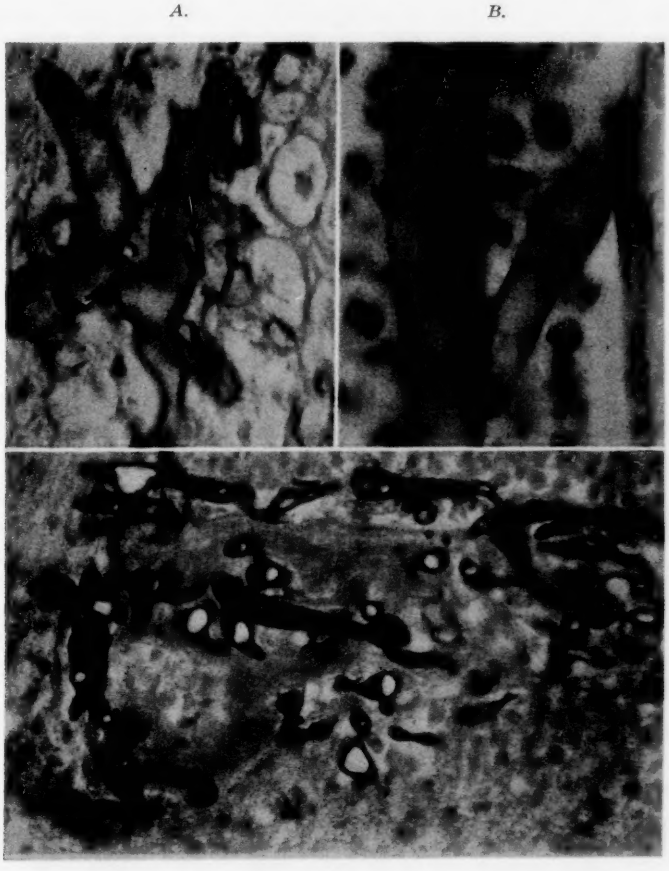


Fig. 6.—*A*, Mucormycosis. Tissue from biopsy of ulcer of palate. Broad, branching, nonseptate hyphae in perineurial lymphatic; nerve fibers to right. *Rhizopus arrhizus* was cultured from this lesion. (Hexatoxylin and eosin.) *B*, Mucormycosis. Branching hypha in blood vessel. (Hematoxylin and eosin.) *C*, Mucormycosis. Hyphae in and around a thrombosed cerebral vessel. Gomori's methenamine-silver nitrate technique.

PENICILLIOSIS

In sections Penicillium is much like Aspergillus. In the one case observed by the writer, as a pulmonary lesion, hyphae were indistinguishable from those of Aspergillus, but Penicillium was demonstrated on culture.

SPOROTRICHOSIS

The Fungus.—*Sporotrichum schenckii*, in tissue, is a cigar-shaped or oval, gram-positive organism 4 to 5 μ in greatest dimension. It is often within the macrophages of lesions of experimentally infected mice. Large asteroid forms, with peripheral radiating spicules, have been reported in human tissues.

The Inflammatory Response.—Tissue from human lesions contains pus, caseation necrosis, or lymphocytic and giant cells. Organisms are seldom demonstrated in human lesions, except in the rare, fatal, generalized cases.

Clinical Correlation.—The clinical picture of a primary ulcer with modules or ulcers along the draining lymphatics is highly characteristic. With a biopsy failing to show organisms, cultures should be obtained; and the diagnosis may be made in a few days, as the organism grows rapidly. To demonstrate the tissue form of the organism, either pus or a suspension of the fungus is injected intraperitoneally into a mouse and sections are made of nodules of the peritoneal wall when the mouse is killed after two weeks.

MADUROMYCOSIS

The Fungus.—Several genera and species are causative agents. The fungus is usually seen in the form of a grain or granule, representing a mycelium or colony of the fungus (Fig. 7). The granules are easily seen with the naked eye, as black, brown, or gray flecks. In tissue sections the granules stain reasonably well with hematoxylin and eosin, and the hyphae and spores of the granule are noted. In contrast to the hyphae of the granules of actinomycosis and nocardiosis, the hyphae of maduromycosis are broad, and stain well with the PAS stain. Occasionally, brown pigment may be seen. In infection with *Monosporium apiospermum* (Fig. 7) the granules are larger than those of actinomycosis, and a central hyphal mycelium presents a peripheral spore-bearing portion. In some infections only spores are present in the granules. Culture is required for the identification of the causative fungus, though the grain of *Monosporium apiospermum* (*Allescheria boydii*) is identifiable in tissue sections.

The Inflammatory Response.—The granule lies within an abscess (Fig. 7) which in turn is surrounded by chronic inflammatory and scar tissue.

Clinical Correlation.—Maduromycosis is usually a disease of a lower extremity, resulting from inoculation of organisms from the soil into the soft tissues. Sinus formation and osteomyelitis commonly develop.

RHINOSPORIDIOSIS

The Fungus.—*Rhinosporidium seeberi* grows as a spore the size of a red blood cell and also as a developing form of larger size. Sporangia may reach a diameter of 350 μ (Fig. 8). Staining with hematoxylin and eosin is satisfactory.

The Inflammatory Response.—This is usually a chronic, round-celled one, rich in plasma cells.

Clinical Correlation.—Tissue obtained by biopsy usually comes from nasal polyps, pharynx, larynx, conjunctiva, skin, penis, vagina, or rectum.

CHROMOBLASTOMYCOSIS

The Fungus.—In lesions, the organism, which may be one of several species, is a rounded brown body, 6 to 12 μ in diameter. Within the organism there are septa for division.[23] The brown bodies often form clusters (Fig. 8). The bodies

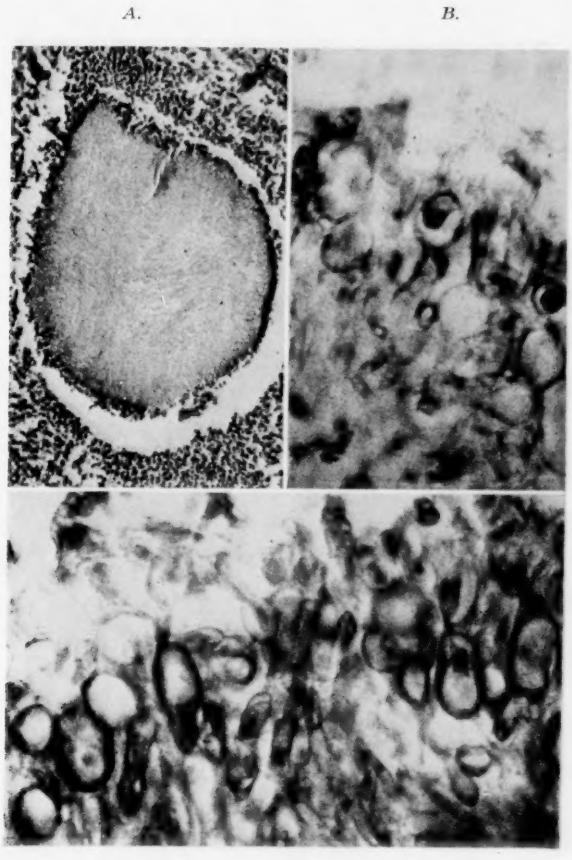


Fig. 7.—*A*, Madurомycosis. Granule surrounded by pus cells. (Hematoxylin and eosin.) *B*, Madurомycosis. Edge of granule. Portions of two rounded cells are gram-positive, but the Gram stain is not helpful with granules of madurомycosis as it is with granules of actinomycosis. Contrast this with Fig. 1. (MacCallum bacterial stain.) *C*, Madurомycosis. Edge of granule. Both spores and hyphae are sharply delineated by this method. (Periodic acid-Schiff stain.) (From Anderson, W.A.D.: Pathology, ed. 2, The C. V. Mosby Co., 1953; in Chapter 15 by Roger D. Baker.)

are so prominently brown in ordinary sections that no special staining is necessary. Culture is required for exact identification of the causative fungus. A related fungus infection has recently been described.[24]

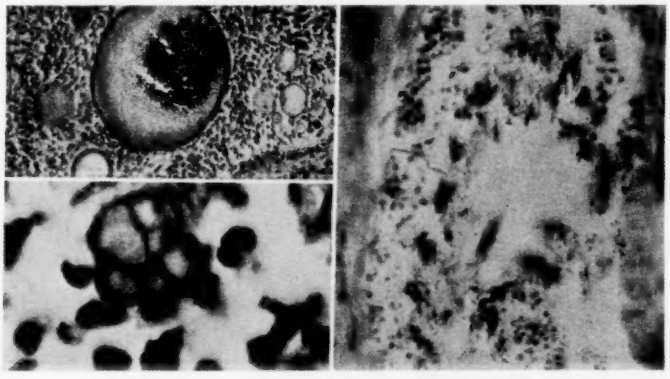
The Inflammatory Response.—The brown bodies are found within giant cells or miliary abscesses of the skin or subcutaneous tissues.

Clinical Correlation.—The infection is usually on an extremity, and the chronic verrucous dermatitis persists for years.

THE DERMATOMYCOSES

The Fungi.—The fungi occur as hyphae or chains of spores. None of the large, complicated spores are seen in tissues such as develop in cultures of Trichophyton, Epidermophyton, and Microsporum. The fungi are visible in hematoxylin and eosin preparations, and in methylene blue (Fig. 8), Giemsa, and PAS stains.

The Inflammatory Response.—The organisms are present in hair or nails without inflammatory response (Fig. 8). In deep infections, which are rare, there may be suppuration and giant-cell response.



A. *B.* *C.*

Fig. 8.—*A*, Rhinosporidiosis. Conjunctiva. Large central sporangium containing endospores. (Hematoxylin and eosin.) *B*, Chromoblastomycosis. A cluster of brown, septate organisms is surrounded by pus cells. (Hematoxylin and eosin.) *C*, Tinea capitis. Minute spores lie between the walls of the hair follicle and the fragmented hair shaft. (Methylene blue stain.) (From Anderson, W.A.D.: Pathology, ed. 2, The C. V. Mosby Co., 1953; in Chapter 15 by Roger D. Baker.)

Clinical Correlation.—Fingernail clippings may be embedded in paraffin, sectioned, and stained for the organisms. In tinea capitis, the organisms lie in the hair follicle and also within the hair shaft. In tinea favosa the scutula are found to consist of abundant mycelial masses. In tinea corporis, organisms are usually not demonstrable in section though rarely they may be seen in the horny layer or within vesicles.

TINEA VERSICOLOR

The Fungus.—Hyphae and rows of spores occur in the stratum corneum, staining with hematoxylin or methylene blue.

The Inflammatory Response.—There is usually none.

Clinical Correlation.—The organism occurs in fawn-colored patches on the skin surface, especially over the chest.

OTOMYCOSIS

The Fungus.—*Aspergillus*, with heads, are readily seen in hematoxylin and eosin and in Gridley stains.

The Inflammatory Response.—There is usually little or none, though scratching may induce chronic dermatitis of the ear canal; and the fungus may be secondary to other conditions, such as eczema.

Clinical Correlation.—In pieces of ear wax embedded and sectioned like tissue, bacteria and fragments of keratinized epidermis are found, in addition to the fungus.

STAINING PROCEDURES

1. *MacCallum-Goodpasture Stain for Gram-Positive and Gram-Negative Bacteria in Tissues.*—Any well-fixed tissue may be used. Cut thin paraffin sections.

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SOLUTIONS

Goodpasture's Stain

Basic fuchsin	0.59 Gm.
Aniline	1.00 c.c.
Phenol crystals (melted)	1.00 c.c.
Alcohol, 30 per cent	100.00 c.c.

Gram's Iodine

Iodine	1.00 Gm.
Potassium iodide	2.00 Gm.
Distilled water	300.00 c.c.

Sterling's Gentian Violet Stain

Gentian violet (crystal violet)	5.00 Gm.
Absolute alcohol	10.00 c.c.
Aniline	2.00 c.c.
Distilled water	88.00 c.c.

Picric Acid Solution

A saturated aqueous solution of picric acid.

TECHNIQUE

1. Deparaffinize to distilled water.
2. Place in Goodpasture's stain for 15 minutes.
3. Wash in distilled water.
4. Differentiate in full strength formalin for a few minutes until section becomes pink.
5. Wash in distilled water.
6. Counterstain in saturated aqueous picric acid for 3 to 5 minutes.
7. Wash in water.
8. Differentiate in 95 per cent alcohol for 3 to 5 minutes.
9. Wash in water.
10. Stain in Sterling's gentian violet solution for 3 minutes.
11. Wash in water.
12. Place in Gram's iodine solution for 1 minute.
13. Blot dry.

14. Place in a solution of equal parts of aniline and xylene, 2 changes.
15. Xylene, 2 changes.
16. Mount in Permount or Clarite.

RESULTS

Gram-positive organisms will be blue; gram-negative organisms, red; background, purplish.

2. *Gridley Fungus Stain.*—Cut paraffin sections at 6 μ from any well-fixed tissue.

SOLUTIONS

Chromic Acid Solution

4 per cent aqueous solution of chromic acid.

Coleman's Feulgen Reagent

Dissolve 1 Gm. of basic fuchsin in 200 ml. of boiling water; filter, cool, and add 2 Gm. of potassium metabisulfite and 10 ml. of normal hydrochloric acid. Let bleach for 24 hours, and then add 0.5 Gm. of activated carbon (Nordit), shake for about 1 minute, and filter through coarse paper. The filtrate should be colorless.

Normal Hydrochloric Acid

Hydrochloric acid, sp. gr. 1.19	83.5 c.c.
Distilled water	916.5 c.c.

Sodium Metabisulfite Solution

10 per cent aqueous solution of sodium metabisulfite.

Sulfurous Rinse

10 per cent sodium metabisulfite	6.0 c.c.
Normal hydrochloric acid	5.0 c.c.
Distilled water	100.0 c.c.

Aldehyde Fuchsin Solution

Basic fuchsin	1.0 Gm.
Alcohol, 70 per cent	200.0 c.c.
Paraldehyde	2.0 c.c.
Concentrated hydrochloric acid	2.0 c.c.

Allow to stand at room temperature for 3 days until solution turns deep blue. Store in refrigerator.

Metanil Yellow

Metanil yellow	0.25 Gm.
Distilled water	100.00 c.c.
Glacial acetic acid	0.25 c.c.

TECHNIQUE

1. Deparaffinize and bring sections to distilled water as usual.
2. Place in 4 per cent chromic acid for 1 hour.
3. Wash in running water for 5 minutes.
4. Place in Coleman's preparation of Feulgen reagent for 15 minutes.
5. Rinse in 3 changes of sulfurous acid rinse.
6. Wash for 15 minutes in running tap water.

7. Place in aldehyde-fuchsin solution for 15 to 30 minutes.
8. Rinse off excess stain with 95 per cent alcohol.
9. Wash in water.
10. Counterstain lightly in metanil yellow solution.
11. Wash in water.
12. Dehydrate in graduated alcohols, clear in xylene, mount in Permount.

RESULTS

Mycelia will be deep blue; conidia, deep rose to purple; background, yellow; elastic tissue and mucin also stain deep blue.

3. *Periodic Acid-Schiff Reaction.*—Cut paraffin sections at 6 μ from formalin or Zenker-fixed tissue.

SOLUTIONS

Periodic Acid Solution

Periodic acid crystals	0.5 Gm.
Distilled water	100.0 c.c.

Schiff's Leucofuchsin Solution

Dissolve 1 Gm. of basic fuchsin in 200 c.c. of distilled water. Bring to boil. Cool to 50° C. Filter and add 20 c.c. of normal hydrochloric acid. Cool further and add 1 Gm. of anhydrous sodium bisulfite. Keep in the dark. The fluid may take two days to become straw-colored; then it is ready for use. Store in refrigerator.

Normal Hydrochloric Acid

Hydrochloric acid, concentrated, sp. gr. 1.19	83.5 c.c.
Distilled water	916.5 c.c.

Sulfurous Acid Rinse

10 per cent sodium bisulfite ($NaHSO_3$)	6.0 c.c.
Normal hydrochloric acid	5.0 c.c.
Distilled water	100.0 c.c.

TECHNIQUE

1. Bring sections to distilled water in the usual way.
2. Periodic acid solution for 5 minutes.
3. Rinse in distilled water.
4. Place in Schiff's leucofuchsin solution for 15 minutes.
5. Rinse in 3 changes of sulfurous acid rinse for 2 minutes each.
6. Wash in running tap water for 10 minutes.
7. Counterstain with Harris hematoxylin for 1 minute.
8. Differentiate in acid alcohol (2 or 3 quick dips).
9. Wash in running tap water until nuclei are clear blue.
10. Dehydrate in graduated alcohols, clear in xylene, and mount in Permount.

4. *Mayer's Mucicarmine Stain.*—Cut paraffin sections at 6 μ from any well-fixed tissue. Use control slide.

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SOLUTIONS

Picric Acid Solution

Picric acid, saturated solution	100.0 c.c.
Glacial acetic acid	5.0 c.c.

Weigert's Iron Hematoxylin

Solution A

1 per cent hematoxylin in 95 per cent alcohol.

Solution B

Ferric chloride, 29 per cent aqueous	4.0 c.c.
Distilled water	95.0 c.c.
Hydrochloric acid	1.0 c.c.

Working Solution

Equal parts of solutions A and B. Prepare fresh.

Metanil Yellow Solution

Metanil yellow	0.25 Gm.
Distilled water	100.00 c.c.
Glacial acetic acid	0.25 c.c.

Mucicarmine Stain

Carmine (alum lake)	1.0 Gm.
Anhydrous aluminum chloride	0.5 Gm.
Distilled water	20.0 c.c.

Mix stain in a small flask and heat over small flame until solution becomes deep red (approximately 2 minutes). Add 80 c.c. of 50 per cent alcohol. Filter each time before use. (Stain is usable immediately and is good for several days, giving best results at 24 to 48 hours.)

TECHNIQUE

1. Deparaffinize and bring sections to water as usual.
2. Treat sections for 30 minutes in saturated aqueous picric acid solution.
3. Wash in running water until clear.
4. Place slides on rack and pour on freshly prepared Weigert's hematoxylin for 4 minutes.
5. Wash in tap water.
6. Stain in metanil yellow solution for 1 minute.
7. Rinse in distilled water.
8. Place in mucicarmine stain for 30 minutes to 1 hour or longer, check control slide microscopically for staining time.
9. Rinse quickly in 95 per cent alcohol.
10. Dehydrate in absolute alcohol, clear in xylene, mount in Permount.

RESULTS

Mucin will be deep rose to red; nuclei, black; other tissue elements, yellow.

5. *A Stain for Fungi in Tissue Sections and Smears, Using Gomori's Methenamine - Silver Nitrate Technique.*—Cut paraffin sections at 6 μ from any well-fixed tissue.

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SOLUTIONS

1. Five per cent aqueous chromic acid (chromium trioxide, CrO_3).
2. Stock methenamine-silver nitrate solution: Add 5 ml. of 5 per cent silver nitrate to 100 ml. of 3 per cent methenamine, U.S.P. grade $(CH_2)_6N_4$. A white precipitate forms but immediately dissolves on shaking. The clear solution remains usable for months at refrigerator temperature.
3. One per cent aqueous sodium bisulfite ($NaHSO_3$).
4. Five per cent aqueous borax, U.S.P. grade ($Na_2B_4O_7.10H_2O$).
5. One-tenth per cent aqueous gold chloride ($AuCl_3.HCl.3H_2O$). May be used repeatedly.
6. Two per cent aqueous sodium thiosulfate ($Na_2S_2O_3 5H_2O$).

TECHNIQUE

1. Deparaffinize sections and bring to distilled water as usual. Smears are prepared on albumin-treated slides and fixed in 95 per cent alcohol.
2. Hydrated sections and smears are oxidized in 5 per cent chromic acid for 1 hour, washed in running tap water for 10 minutes, and then treated in sodium bisulfite for 1 minute to remove any residual chromic acid. They are then washed in tap water for 5 minutes and finally in 3 changes of distilled water.
3. Silver at 45° to 50° C. in a working solution prepared by adding 25 ml. of stock methenamine-silver nitrate to an equal portion of distilled water containing 1 to 2 ml. of 5 per cent borax. Fungi and mucin will begin to stain at the end of 25 to 30 minutes and will be adequately stained at the end of an hour. Slides are then rinsed in distilled water 2 or 3 times.
4. Tone in 0.1 per cent gold chloride for 5 minutes. This will also bleach the background. Rinse in distilled water.
5. Remove unreduced silver by treating with 2 per cent sodium thiosulfate for 1 or 2 minutes and, after washing thoroughly, counterstain if desired; using safranin if a red nuclear stain is desired, or a light hematoxylin-eosin combination if tissue detail is important.
6. Dehydrate, clear, and mount as usual.

RESULTS

Fungi are sharply delineated in black with the inner parts of mycelia and hyphae staining an old rose as a result of toning in gold. Mucin also assumes a rose-red color as a result of toning.

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SEROLOGIC REACTIONS IN COCCIDIOIDOMYCOSIS

CHARLES E. SMITH, M.D., AND MARGARET T. SAITO, B.S.
BERKELEY, CALIF.

From the School of Public Health, University of California, Berkeley, Calif.

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EXPERIENCE with coccidioidal serologic tests during the past seventeen years has indicated their values and limitations in the diagnosis and prognosis of coccidioidomycosis. A qualitative precipitin test and a quantitative complement fixation (C.F.) test have been necessary for recently acquired infections. For long-standing infections only the quantitative C.F. test has been useful. A report in 1950[4] has summarized the background of these tests, the techniques used, and initial results in their application. A second publication[5] has extended this experience. Beginning in 1949 a collaborative study was instituted to determine feasibility of widespread use of these tests. These results have been reported recently.[6] They have revealed that different techniques of quantitative C.F. tests affect the absolute titers of the positive sera but indicate that their prognostic patterns are consistent. Moreover, they strongly suggest that a lyophilized positive control serum may be adjusted to a "critical" titer which is a common denominator for these differing techniques. The number of collaborating laboratories has been expanded from four to twelve. Their promising results indicate that the principal problem is the feasibility of the standardized positive serum in terms of the prognostic application of the tests. Commercial distribution of the diagnostic materials has been deferred pending the outcome of these studies. However, with this availability hopefully imminent, it is appropriate to review the coccidioidal serologic tests in this Symposium on mycoses.

TECHNIQUE OF SEROLOGIC TESTS

Filtrates of liquid cultures, fungus suspensions, and extracts have all been used as coccidioidal antigens. Autoclaving does not seriously deteriorate antigen for precipitins but it ruins antigen for fixation of complement. To facilitate preparation we have used filtrates of the liquid asparagine synthetic medium of skin-testing "Coccidioidin" inoculated with multiple strains of *Coccidioides immitis*. These filtrates contain aqueous Merthiolate in a final concentration of 1:10,000 and are quite stable even at room temperature.

The antigen for precipitin tests is relatively easily prepared. Antigens of inadequate potency can be concentrated behind collodion-coated alundum filters to standard strength.

Preparation of the antigen for C.F. tests is more difficult. Flasks of the same lot of media inoculated with the same suspension and incubated simultaneously may vary greatly in potency and in anticomplementary quality. Concentration

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has not proved feasible. Thus the contents of each flask must be tested before being pooled. Fortunately, satisfactory antigens remain potent indefinitely.

The precipitin test is performed in 70 by 7 mm. test tubes by mixing undiluted serum with undiluted and 1:10 antigen and a control of uninoculated medium. The serum and the antigens are each used in 0.2 ml. volumes and contain aqueous Merthiolate to a final concentration of 1:10,000. The mixtures are placed in a 37° C. incubator and read daily for five days. Only a definite button or flake, readily spun from the bottom by sharply flicking the tube, is considered positive. This is much more objective than attempting to read a ring of precipitate at the antigen-serum junction.

Most precipitates form in one or two days, but occasionally they do not develop for three or four days. Therefore, a five-day holding period is appropriate. The Merthiolate prevents contamination unless the serum becomes grossly contaminated. Contamination, hemolysis, or lipemia interfere with the precipitin tests. Formerly 1:20, 1:40, and 1:100 antigen dilutions also were used. However, since the tests are qualitative rather than quantitative, these three antigen dilutions afford no significant additional knowledge. In one-seventh of sera with demonstrable precipitins, only the undiluted antigen evokes a positive test. One per cent of sera with precipitins show prezoning, the undiluted antigen being negative and the 1:10 antigen positive, so 1:10 antigen also should be used. However, the 1:10 antigen dilution never has shown prezoning with respect to higher dilutions.

The quantitative C.F. test can be performed with the technique the laboratory is accustomed to use. However, variations in methods affect the titer and, as will be noted, titer has been shown to have important prognostic value. It is hoped that a positive serum control can be adjusted to the critical titer so that, by whatever method used, results above that level will indicate severe disease, while below it the prognosis will be much more favorable. The method of C.F. by which 39,500 sera have been studied and reported[5] may be outlined as follows.

Primary incubation for 2 hours at 37° C. binds 0.25 ml. of each dilution of serum, 0.25 ml. of antigen, and 2 units of complement in 0.5 ml. The hemolytic system consists of 0.5 ml. of a 2 per cent suspension of sheep erythrocytes sensitized with 2 units of amboceptor. The hemolytic system is incubated another hour with the serum and an initial reading is made. The final reading is taken after overnight refrigeration. If overnight 4° C. binding is substituted for 2-hour 37° C. primary incubation, the titer is increased one serial dilution and the test also seems more sensitive.[5] Thus in patients with primary infections one-third of sera having precipitins but which do not fix complement with 2-hour 37° C. binding do so with overnight icebox binding.

Because of the variation between tests, it is desirable to freeze positive sera and repeat a previous test simultaneously with new specimens. This enables one to detect ominous rises or reassuring declines in titers.

SPECIFICITY

The coccidioidal serologic tests, like the intradermal test with Coccidioidin, do not cross-react with viral, rickettsial, bacterial, or spirochetal infections.

There is no evidence of cross reaction in dermatophytosis, actinomycosis, or cryptococcosis. Again, as in the skin testing, there is irregular cross reaction in histoplasmosis,[3,4] and presumably, blastomycosis. The experiences of Campbell[1] and Salvin[3] have indicated that coccidioidal antigens are somewhat less specific in precipitin than in C.F. tests but that they are more specific than are histoplasmal and blastomycotic antigens in coccidioidal infections. Our own recent experience in a three-man epidemic of histoplasmosis acquired in a Mexican bat-cave has borne out these views. Characteristically, the homologous antigen results in a significantly higher titer in the serologic tests. Furthermore, since histoplasmosis and blastomycosis are associated with damper, and coccidioidomycosis with more arid areas, travel and residence histories also assist in evaluating possibility of cross reactions in acute, recently acquired infections. However, one may occasionally be uncertain and have to depend upon the isolation of the fungus for differentiation.

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ACCURACY OF DIAGNOSTIC SEROLOGIC TESTS OF COCCIDIOIDAL INFECTION

The greatest diagnostic value of coccidioidal serologic tests is in progressive, disseminated coccidioidomycosis. During seventeen years of experience the sera of only 2 of 722 patients with disseminated disease[5] have had neither precipitins nor complement-fixing antibodies. In 2 other patients with disseminated disease whose sera failed to show precipitins, complement was not fixed by 2-hour 37° C. binding but was demonstrable by overnight 4° C. binding. One can virtually eliminate the diagnosis of progressive disseminated coccidioidal disease if precipitins and complement-fixing antibodies are absent. One should hesitate at ruling out as coccidioidal an isolated extrapulmonary lesion, even of the meninges, on the basis of negative serologic findings. However, progressive coccidioidal processes almost certainly have been diagnosable serologically.

The less severe the infection, the less the likelihood of being able to make the diagnosis serologically. Repeated tests of serum specimens have established the diagnosis in 92 per cent of 730 soldiers with primary infections of sufficient severity as to require hospitalization. However, the tests have been positive only in 7 per cent of those with recently acquired asymptomatic primary infections. One-third of the sera of patients with primary infections and positive serologically can be diagnosed only by precipitin tests.[5]

Even when coccidioidal pulmonary residuals remain, there is strong probability that the serologic tests will be negative. In patients with proved coccidioidal pulmonary cavitation, 40 per cent have equivocal or negative tests. Patients with asymptomatic, noncavitary pulmonary lesions are especially difficult diagnostic problems. The proportion of those patients diagnosable serologically has never been determined, but is relatively small. The serologic tests are valuable in that a *positive* test establishes the diagnosis, but a negative test does not eliminate it. Fortunately, diligent culturing usually can establish etiology of coccidioidal pulmonary cavities. However, for noncavitary pulmonary residuals, exploratory thoracotomy frequently is necessary. Moreover, one should continue to be on one's guard for dual infections, notably tuberculosis, and for concomitant malignancy.

SIGNIFICANCE OF TIME RELATIONSHIPS OF DIAGNOSTIC HUMORAL ANTIBODIES

Dermal sensitivity to Coccidioidin is established before diagnostic humoral antibodies are detectable in primary nondisseminated disease.[4,5] Therefore, since Coccidioidin neither sensitizes nor stimulates humoral antibodies, even in reactors, a Coccidioidin skin test should be performed prior to serologic testing. The serologic tests should be deferred in nonreactors with possible primary infections unless dissemination is suspected.

The times of appearance of precipitins and complement-fixing antibodies in patients with nondisseminated primary coccicioidal infections are contrasted in Fig. 1. While dermal sensitivity to 1:100 Coccidioidin is present in five-sixths

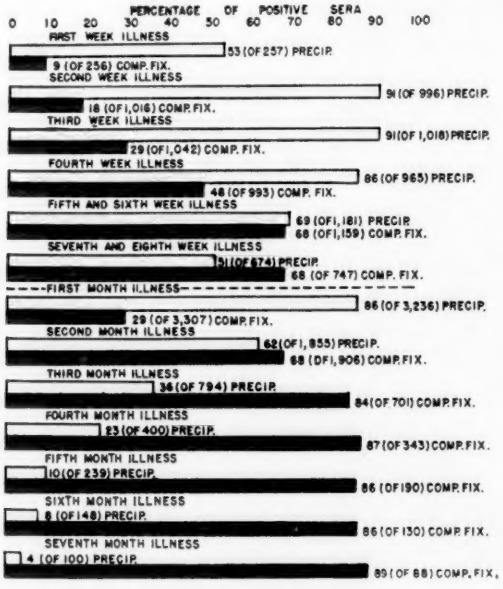
PERCENTAGE OF POSITIVE SERA
0 10 20 30 40 50 60 70 80 90 100
FIRST WEEK ILLNESS
53 (OF 257) PRECIP.
9 (OF 256) COMP. FIX.
SECOND WEEK ILLNESS
91 (OF 996) PRECIP.
18 (OF 1,016) COMP. FIX.
THIRD WEEK ILLNESS
91 (OF 1,018) PRECIP.
29 (OF 1,042) COMP. FIX.
FOURTH WEEK ILLNESS
86 (OF 965) PRECIP.
48 (OF 993) COMP. FIX.
FIFTH AND SIXTH WEEK ILLNESS
69 (OF 1,181) PRECIP.
68 (OF 1,159) COMP. FIX.
SEVENTH AND EIGHTH WEEK ILLNESS
51 (OF 674) PRECIP.
68 (OF 747) COMP. FIX.
FIRST MONTH ILLNESS
86 (OF 3,236) PRECIP.
29 (OF 3,307) COMP. FIX.
SECOND MONTH ILLNESS
62 (OF 1,855) PRECIP.
68 (OF 1,906) COMP. FIX.
THIRD MONTH ILLNESS
36 (OF 794) PRECIP.
84 (OF 701) COMP. FIX.
FOURTH MONTH ILLNESS
23 (OF 400) PRECIP.
87 (OF 343) COMP. FIX.
FIFTH MONTH ILLNESS
10 (OF 239) PRECIP.
86 (OF 190) COMP. FIX.
SIXTH MONTH ILLNESS
8 (OF 148) PRECIP.
86 (OF 130) COMP. FIX.
SEVENTH MONTH ILLNESS
4 (OF 100) PRECIP.
89 (OF 88) COMP. FIX.

Fig. 1.—Percentage distribution of precipitins and complement-fixing antibodies in sera of patients with primary nondisseminated coccidioidomycosis by duration of illness. (From Smith, Saito, and Simons,[5] by permission of J.A.M.A.)

of patients during the first week of symptoms,[7] precipitins are detectable in the sera only of one-half, and complement is fixed by the two-hour 37° C. binding in one-tenth.[5] Precipitins peak in the second and third week when they are present in nine-tenths of patients' sera. Occasionally they develop as long as six weeks after onset. However, as reversions from positive to negative begin in the third week, not even all patients with sera manifesting fixation of complement will show precipitins. One recalls that only 1 per cent are nonreactors to 1:100 Coccidioidin in the third week, and thereafter all patients with nondisseminated primary infections react to this skin test.

Complement-fixing antibodies never attain the proportions of the precipitins. The maximal ratio is two-thirds at the end of the second month. Sera

of patients may continue to become positive in the third month, but in others previously positive, sera lose complement-fixing capacities within the second month. The continuing increase in proportion of C.F. seen in Fig. 1 during later months merely reflects the fact that the sample has been biased. Sera of more seriously ill patients have been repeated for the prognostic values of the quantitative C.F. test, and therefore the results have been distorted. The time of disappearance of complement-fixing antibodies cannot be quantitated from our data because of this selection of our "initial" and "repeat" specimens in later months from patients with more severe illnesses. Certainly, complement-fixing antibodies persist longer than do precipitins and independently of the latter. Indeed, in nearly half the serum specimens of patients with primary infections, which contained both precipitins and complement-fixing antibodies, the latter increased in titer as the precipitins vanished. After several years the sera of most patients no longer fix complement to a diagnostic level. Fortunately, from the viewpoint of diagnosing coccidioidal pulmonary residuals with and without cavitation, sometimes sera of these patients continue to fix complement. Occasionally sera of these patients will fix complement after the patient does not react to the 1:100 or even 1:10 Coccidioidin skin test. These purported failures to react to Coccidioidin are complicated by omissions of reading the skin tests at both 24 and 48 hours. However, even if his Coccidioidin skin test is negative, a patient with a "classical" thin-walled pulmonary cavity and with a plausible residence history may warrant a coccidioidal complement fixation test. Complement-fixing antibodies may persist for many years and at a low or moderate stable titer are not indicative of "activity" of infection.

As Fig. 1 indicates, precipitins disappear much more predictably. They begin to be lost in the third week of illness. The loss is rapid after the fourth week so that by six months less than one-tenth of patients with primary diseases have sera with precipitins. Thus, in diagnosis of patients with pulmonary residuals with or without cavitation, the precipitin test is inappropriate. Although precipitins may persist rarely into the second and even third year of nondisseminated coccidioidal disease, their presence bespeaks a fairly recent infection. Their relatively rapid disappearance accounts for the fact that in 23 per cent of nearly 5,600 patients with nondisseminated primary infections diagnosable serologically, only the C.F. test has been positive. Thus both precipitin and C.F. tests are indispensable in the serologic diagnosis of primary infections.

Although there is no evidence of predilection to dissemination with persistence of precipitins, they do imply "activity" of infection and appear to occur longer in patients undergoing dissemination. As in nondisseminated infections, precipitins are infrequent in serum after six months. They are found only occasionally after a year, but one patient had precipitins in the ninth year after infection and dissemination. Bespeaking their early disappearance, in one-half of over 700 patients with disseminated disease sera fixed complement but did not contain precipitins. Only in a few patients who undergo the rupture of a pulmonary cavity to the pleural space and resultant hydropneumothorax have we noted precipitins reappear after they had vanished.[4]

TABLE I. PERCENTAGE DISTRIBUTION OF MAXIMAL TITERS OF COMPLEMENT-FIXING ANTIBODIES IN SERA OF VARIOUS TYPES OF COCCIDIOIDAL DISEASES

MAXIMUM TITER OF COMPLEMENT FIXATION	NONDISSEMINATED INFECTION			DISSEMINATED INFECTION				
	UNCOMPLICATED PRIMARY	PULMONARY RESIDUAL	PULMONARY CAVITATION	SINGLE LESION (EXCEPT MENINGITIS)	MENINGITIS	EXTENSIVE (EXCEPT MENINGITIS)	UNKNOWN	ALL DISSEMINATIONS
	N = 3,154	N = 271	N = 577	N = 141	N = 136	N = 291	N = 141	N = 709
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
2*	41.7	72	54	18	7	1	1	6
4†	26.9	20	28	18	11	2	2	7
8	16.8	5	10	27	12	5	4	11
16	9.3	2	6	22	21	9	19	15
32	3.9	1	2	8	27	16	35	20
64	0.7	0	(0.4)	3	10	28	24	18
128	0.6	0	0	3	7	21	11	13
256	0.1	0	0	1	5	18	4	10
<32	94.7	99	98	85	51	17	26	39
<64	98.6	100	99.6	93	78	33	61	59

*2 = 1 in 2. †4 = 1 in 4.

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PROGNOSTIC SIGNIFICANCE OF TITER OF COMPLEMENT FIXATION

Table I and Fig. 2 indicate that the milder the coccidioidal infection, the lower the titer of the quantitative C.F. test. One recalls that in the sera of at least one-third of the patients with serologically diagnosable primary nondisseminated infections, complement is never fixed and only precipitins are positive. When complement is fixed, in nearly half (Table I) only the initial (1:2) serum dilution is positive. The complement-fixing sera of nearly 95 per cent of the patients with primary infections do not exceed at 1:16 dilution when the method of 2-hour 37° C. binding is used.

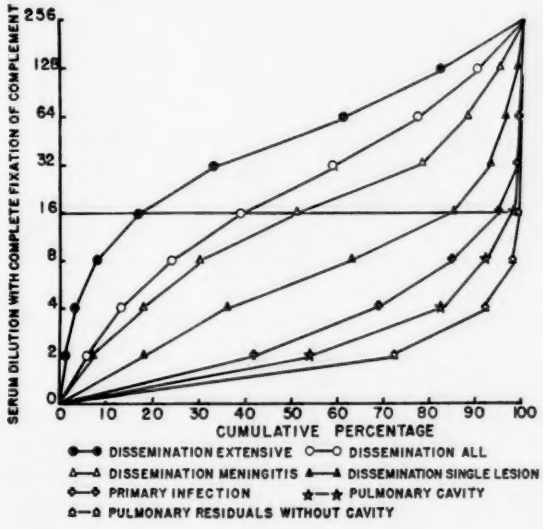

Fig. 2.—Maximal titers of complement-fixing antibodies in sera from various types of coccidioidal diseases, expressed as cumulative percentages. Data from Table I. (From Smith, Saito, and Simons,[5] by permission of J.A.M.A.)

Patients having coccidioidal pulmonary residuals without cavities and even with cavities are in the same category as those with current primary nondisseminated infections. The cumulative curves of the complement-fixing titers of their sera are even lower. Ninety-nine per cent of those with noncavitary residuals and 98 per cent of those with cavities have sera with titers no higher than 1:16. These findings reflect that these pulmonary lesions are merely the legacies of primary infections and in an immunologic sense these infections are focalized.

In contrast, patients with disseminated disease demonstrate that the more severe the coccidioidal infection, the higher the titer at which sera will fix complement. Martin[2] has reported the same relationship in blastomycosis. Sixty-one per cent of 709 patients with disseminated coccidioidal infections have had titers of sera exceeding 1:16. The cumulative curves of the titers seen in Fig. 2 show a progressive rise from the sera of patients with a single extrapulmonary lesion, with only 15 per cent in excess of 1:16, to 83 per cent in excess of 1:16 in

sera of patients with extensive lesions. Patients with meningitis have complement-fixing titers of serum between these two. Sometimes meningitis is the only extrapulmonary lesion detectable, while in other patients the meningeal involvement is only one site of generalized dissemination.

When one is following serologically the course of a patient with coccidioidal infection, a rising titer of complement fixation connotes increasing severity. A regression in titer is reassuring. With 2-hour 37° C. binding, a complement fixing level about 1:16 makes one apprehensive of the possibility of dissemination. However, comparisons have shown that this "critical titer" varies in accordance with the methods of performing the quantitative C.F. tests. Therefore, if a positive control serum can be adjusted to approximate this "critical titer," it can serve as a common denominator.

COMPLEMENT FIXATION BY SPINAL FLUID AND OTHER BODY FLUIDS

As has been discussed already, serum may fix complement at only a very low titer in patients with coccidioidal meningitis. Indeed, for some time the results may be equivocal. Also, recovery of the fungus from the spinal fluid may be difficult. However, with increased white cells, mostly lymphocytes, in the cerebrospinal fluid, accompanied by increased protein and reduced sugar, coccidioidal meningitis is virtually certain if one can prove a concurrent coccidioidal infection. Definite fixation of complement by spinal fluid occurs only in coccidioidal meningitis. Cerebrospinal fluid of patients with serum titers of 1:246 in C.F. tests but who do not have meningitis have not fixed complement. Spinal fluid does not always fix complement even when meningitis is coccidioidal. Of 92 patients with coccidioidal meningitis in whom both serum and spinal fluid specimens have been sent for complement fixation tests, 76 per cent have had definite fixation of complement by the spinal fluid. Sometimes repeated specimens have been necessary before progression of the meningitis resulted in positive findings. Usually the titer of the spinal fluid is two serial dilutions lower, though occasionally it may even exceed that of the serum. Precipitins are very rarely detectable in the spinal fluid and never when complement is not fixed. Thus, testing the spinal fluid for precipitins is a useless procedure.

Pleural fluid and ascitic fluid are also positive at times in patients with coccidioidal pleurisy with effusion or with coccidioidal peritonitis. Both precipitins and complement-fixing antibodies may be present, but we never have detected them in these body fluids when absent from the serum. The latter is preferable for diagnostic studies.

SUMMARY

Coccidioidal serologic tests consist of a qualitative precipitin test and a quantitative complement fixation (C.F.) test. The antigens for each of these tests are appropriately standardized "Coccidioidins" comparable to that used for skin testing. The tests are relatively specific, though there is some irregular cross-reaction in the other deep mycoses of histoplasmosis and probably blastomycosis. The tests detect better than 99 per cent of all coccidioidal infections which have undergone dissemination. Repeated tests can diagnose 90 per cent of

symptomatic primary infections. However, even in symptomatic primary infections, failure to detect these diagnostic humoral antibodies does not rule out a coccidioidal diagnosis. They are frequently negative in patients with coccidioidal pulmonary residuals with and without cavitation.

Unless a patient's disease is undergoing dissemination, dermal sensitivity will be established during primary infection before the humoral antibodies have developed. Precipitins develop before complement-fixing antibodies and vanish sooner. Their presence connotes that the infection was acquired in the previous few months, although they may persist longer in patients with disseminated infections. Precipitins are of little diagnostic use in patients with asymptomatic pulmonary residuals or pulmonary cavities because of this time relationship. The titer of precipitins does not have prognostic significance. However, the quantitative determination of complement-fixing antibodies is very important prognostically. The more severe the infection, the higher the C.F. titer. Thus, in at least one-third of patients with primary infections which can be diagnosed serologically, only the precipitins will be positive. In those whose serum fixes complement, nearly half do so only in a 1:2 serum dilution and only 1 in 20 will fix it at a titer greater than 1:16 with the technique we use. However, over three-fifths of all those with disseminated disease exceed this titer as do nine-tenths of those with extensive dissemination. With our technique this 1:16 serum dilution has proved to be a "critical" level. A rising titer is ominous, while a declining titer is reassuring. Complete fixation of complement by spinal fluid is diagnostic of coccidioidal meningitis and occurs in three-fourths of those with this dread complication.

Because variations in C.F. techniques affect the titer, a positive serum control adjusted to the "critical" titer is needed as an index of comparison. Every effort is being made to develop such a material for the commercial distribution which should enable any competent clinical laboratory to perform the coccidioidal serologic tests.

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EVALUATION OF SKIN TESTS AND SEROLOGIC METHODS IN FUNGUS INFECTIONS

DONALD S. MARTIN, M.D., D.P.H.

ATLANTA, GA.

From Communicable Disease Center, U. S. Public Health Service, Department of Health, Education, and Welfare, Atlanta, Ga.

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IT HAS been assumed that the specific immunologic tests used in each of the mycoses will be discussed in other papers of this Symposium; therefore, this presentation is limited to general remarks concerning the evaluation and interpretation of the results of immunologic procedures.

For ease in presentation, this paper is divided into three parts. The first part, "Antigens Used in Immunologic Tests," deals with the various preparations which have been recommended for use in immunologic studies. The second part, "Immunologic Procedures," summarizes briefly the various techniques which can be used and their limitations. The third part, "Practical Application of Immunologic Tests," gives the relative values of immunologic procedures from the standpoint of: (1) determining epidemiologic patterns, (2) finding cases, (3) confirming suspected cases by laboratory examination, (4) evaluating prognosis, and (5) planning therapy.

ANTIGENS USED IN IMMUNOLOGIC TESTS

Two general types of basic materials are used to prepare antigens for use in immunologic tests: (1) filtrates of cultures of the fungus grown in a liquid medium; and (2) fungus cell suspensions of yeast-phase cultures grown on a solid or in a liquid medium.

FILTRATE ANTIGENS

The modern filtrate antigens (Coccidioidin, Histoplasmin, Blastomycin) are filtrates of broth cultures of the fungus growing in the mycelial phase for periods of several months or more. Such filtrates contain all of the soluble metabolic products and other materials which accumulate during the growth period.

A. *Coccidioidomycosis.*—

1. *Coccidioidin:* The filtrate type of antigen has been used exclusively in studies of coccidioidomycosis since yeast-phase cultures of *C. immitis* are difficult to obtain under laboratory conditions. Coccidioidin has been evaluated extensively as a skin-testing, complement-fixing, and precipitating antigen and has proved its value in attaining all five of the objectives listed above.

Studies on the immunology of coccidioidomycosis were facilitated by a number of fortuitous circumstances such as: (1) the geographically concentrated area of Coccidioides infection; (2) the opportunities to perform immunologic studies on individuals whose infections covered the entire range of clinical types from the asymptomatic through the benign, self-limited pulmonary forms of infection to the highly fatal disseminated type of disease; (3) the unique situation brought about by the introduction of large numbers of susceptible individuals into military installations within the hyperendemic area; (4) the centering of most of the laboratory activities in a few cooperating laboratories; and (5) the fact that Coccidioidin probably is the most specific filtrate type of fungus antigen available. Medical mycologists and physicians are indebted to Dr. C. E. Smith and his co-workers not only for their thorough and brilliant investigations of coccidioidomycosis but also for their contributions to the establishment of a pattern which serves as a background for the interpretation of results obtained by immunologic tests in other mycoses.

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The interpretation of the results obtained with Coccidioidin as a skin-testing and serologic antigen have been published by Smith and his group.[52-54] Although Coccidioidin has been used as if it were a single antigen, the data available indicate that it contains different antigenic materials. The skin-testing potency of different lots vary even when identically prepared media are seeded with identical inocula and incubated for identical periods of time on the same shelf in the same incubator.[54] Consequently the potencies of new lots must be standardized by testing the reactions elicited in a group of individuals composed of known reactors, known nonreactors, and equivocal reactors. The presence of more than one antigen in Coccidioidin is suggested by the fact that autoclaving destroys its complement-fixing properties[17,54] without diminishing its effectiveness as a skin-testing and precipitating antigen[17,54,57] and by the observation that a Coccidioidin of "adequate potency for complement fixation may be inferior as antigen for precipitins."[53]

Coccidioidin derivatives: A polysaccharide material precipitated from Coccidioidin is active as a skin-testing and precipitating antigen but this substance does not fix complement with antibodies in human serum.[14,17,18]

The polysaccharide precipitated from Coccidioidin apparently is no more specific as a skin-testing antigen than the original Coccidioidin.[54]

B. *Histoplasmosis.*—

1. *Histoplasmin*: The opportunities for evaluating Histoplasmin have not been as favorable as those which existed for the evaluation of Coccidioidin for the following reasons. (1) *Histoplasma capsulatum* infections, as determined by skin test surveys, occur over wide areas of the United States and other countries. (2) There are relatively few opportunities to study immunologic reactions in large numbers of patients with all forms of the disease. (3) Many laboratories, using different techniques, are involved in the studies of epidemics and of isolated cases. (4) Histoplasmin appears to be the least specific of the filtrate antigens.

As with Coccidioidin, variations in the potencies of different lots occur in spite of intense efforts to prepare them under identical conditions.[20] The extensive cross reactions between Histoplasmin and other similarly prepared fungus

antigens, particularly Blastomycin, present difficulties in the interpretation of skin test results.[10] An increase in the specificity of the skin test reactions, as measured in experimentally infected animals, can be attained by diluting each antigen in accord with its "critical titer," defined as the "minimum amount of antigen which would detect sensitivity in approximately 80 to 90 per cent of animals" experimentally infected with the homologous organisms.[20] Histoplasmin, like Coccidioidin, must be carefully standardized.

Histoplasmin can be used as a complement-fixing antigen for demonstrating antibodies in experimentally inoculated animals[58] and naturally infected humans.[11] Cross reactions with Blastomyces-infected guinea pigs occur, but higher titers usually are obtained with the homologous antigen.

Histoplasmins prepared from different strains of *H. capsulatum* vary greatly in their capacities to fix complement with sera from hyperimmunized rabbits and from patients with proved histoplasmosis.[50] That such variations in potency have their origins in the particular fungus strains is suggested by the observation that new lots of Histoplasmin prepared from the same strains three years later show similar differences.[47]

Histoplasmins also have been used as antigens in precipitin tests.[1,37,40,42]

2. *Histoplasmin derivatives:* Histoplasmin has been used to sensitize collodion particles[43,45] and sheep's erythrocytes[36] for agglutination tests.

A polysaccharide precipitated from Histoplasmin elicits skin test reactions in guinea pigs inoculated experimentally with *H. capsulatum* or with Blastomyces unless the dose, like that of the parent Histoplasmin, is adjusted carefully.[5]

C. *Blastomycosis.*—

1. *Blastomycin:* Filtrate antigens of *Blastomyces dermatitidis* have been prepared in the same manner as those for *C. immitis* and *H. capsulatum*, but such Blastomycins have been used primarily as control materials in serologic and skin test studies in histoplasmosis and have not been used extensively in studies of blastomycosis.

YEAST-PHASE ANTIGENS

Yeast-phase antigens are prepared by culturing the fungus on a solid medium[22,32] or in liquid media.[7,41,56] Such antigens presumably should be more satisfactory than the filtrate antigens since the yeast phase of the fungus more closely resembles the organisms as they appear in infected body tissues.

Yeast-phase organisms grow quite rapidly, permitting use of young cultures; the cells can be washed and most of the extraneous material can be removed.

Yeast-phase antigens, or their derivatives, have been studied in blastomycosis, histoplasmosis, and candidiasis (moniliasis).

A. *Blastomycosis.*—

1. *Yeast-phase antigens:* Yeast-phase antigens are prepared from the growth on a solid medium incubated at 37° C. for approximately 7 days on blood agar[32] or on Kelley's medium.[22] A liquid medium also has been used.[7]

The organisms are washed off the slants with saline, centrifuged, and a suspension containing 0.1 per cent by volume is prepared. For skin testing the suspension is heated at 60° C. for 4 hours, subcultured to insure sterility, and a preservative is added.

For complement fixation tests, a dense suspension of the living organisms in saline is prepared and its anticomplementary properties are titrated. The antigen used in the final test should be no greater than one-fourth of the dose calculated to be anticomplementary.[25]

2. *Yeast-phase derivatives:* In 1953 I studied[29] two types of antigens derived from yeast-phase cells of *Blastomyces dermatitidis:* (1) a protein-containing antigen liberated from the washed cells by sonic vibration; and (2) a non-protein-containing polysaccharide material found in the supernates of untreated saline cell suspensions. The former substance fixed complement with sera from patients with blastomycosis and with sera of hyperimmunized rabbits, whereas the supernatant material fixed complement only with hyperimmune rabbit sera.

The material liberated from *B. dermatitidis* by sonic treatment was much less specific as a skin-testing substance than the supernatant material. Of 12 Histoplasma-infected guinea pigs, 7 reacted strongly to the intracutaneous injection of the sonic filtrate of Blastomyces cells whereas only 1 reacted to the injection of the supernatant material.[29] Similar findings were reported by Dyson and Evans[7] who found that the "somatic antigen extracted from yeast-phase cells did not possess the desired criteria of simultaneous high sensitivity and specificity," whereas a fraction precipitated by ethanol from the supernate of the yeast-phase cultures of Blastomyces not only produced skin reactions in Blastomyces-infected rabbits but failed to elicit reactions in rabbits infected with *H. capsulatum, C. immitis, C. albicans, S. schenckii, and C. neoformans.*

B. *Histoplasmosis.*—

1. *Yeast-phase antigens:* Salvin[40] prepared a yeast-phase antigen by washing cells cultivated in a semifluid medium and reported that it gave consistently higher titers in Histoplasma-infected rabbits than were obtained with a Histoplasmin prepared from a different strain of *H. capsulatum.* None of 8 Blastomyces-infected rabbits fixed complement with the yeast-phase antigen, but low-titered positive reactions occurred with the Histoplasmin antigen. In contrast, a yeast-phase antigen prepared from the growth on glucose-cystine agar slants by Saslaw and Campbell[44] fixed complement with sera from rabbits immunized with *H. capsulatum* and *B. dermatitidis.* The two studies differed in that infected rabbits were used for the former studies and hyperimmunized rabbits were used in the latter.

Ground yeast-phase antigens prepared from washed cells grown on the glucose-cystine media are similar in antigenic properties to the whole yeast-phase antigens but are reported to give more clear-cut end points and to be more stable in respect to the development of anticomplementary properties on storage.[3] Tests with ground antigens on 73 Histoplasmin-sensitive normal individuals showed 10 (13.7 per cent) to fix complement in serum dilutions of 1:10, 1:20, or 1:40, and 6 of 134 nonsensitive individuals to react positively in 1:10 or 1:20 dilutions of serum.[2] Titers up to 1:1,280 were obtained in acute cases of proved histoplasmosis, but, in chronically ill patients, low and even negative titers were obtained.[2]

2. *Yeast-phase derivatives:* Sorensen and Evans[56] reported that an antigen could be obtained from the supernates of yeast-phase broth cultures by zinc

and alcohol precipitation with an accompanying removal of protein. The precipitated fraction fixed complement with sera from Histoplasma-infected rabbits but not with sera from Blastomyces-infected rabbits. A ground-yeast phase antigen and the supernate from which the fraction was prepared reacted with sera from rabbits infected with both fungi.

C. *Candidiasis* (*Moniliasis*).—*C. albicans* grows abundantly in the yeast phase on Sabouraud's agar at room temperature, and saline suspensions can be used as agglutinating antigens[26,31,38] or, if heat-killed, as skin-testing materials.[26]

Immunologic Procedures

The immunologic procedures used in the mycoses are not different from those used in other infectious diseases. However, the antigenic structures of the fungi are quite complex and certain techniques are reviewed in terms of their application to the study of mycotic infections.

Skin Tests.—Skin tests with fungus antigens are performed in the same manner as the tuberculin test. Positive reactions are of the "delayed" type and the tests are read, on the average, 48 hours after injection. Some reactions attain their peaks within 24 hours, whereas others develop more slowly. An indurated area, 5 mm. or more in diameter, is interpreted as a positive reaction.[20,54] Individuals who respond with an immediate "wheal" type of reaction and do not develop induration later probably are not true reactors.[54]

If the skin-testing antigen contains particulate cellular material, such as the case with Blastomyces vaccine, a sterile abscess may develop in the very hypersensitive patient.[33,34] Some investigators attach significance to accurate measurement of the reaction diameter and, in Histoplasmin sensitivity surveys, have recorded the results in the form of histograms in which frequency distributions are plotted in terms of reaction diameters which differ only by 2 mm. increments.[8,21] Since very large variations in the sizes of Histoplasmin skin reactions have been observed in the same individual undergoing repeated skin tests,[39] it is difficult to attach significance to small differences in the diameters of indurated areas which most likely result from unavoidable errors in injecting the skin-testing material. A few people (1.6 per cent) also react to the buffer diluent with indurated areas of 5 mm. or more.[8]

The most common avoidable error is that of failing to take the precaution of using a different syringe for each type of antigen injected.

Complement Fixation Tests.—Any well-controlled and standardized complement fixation procedure may be used to titrate complement-fixing antibodies. It appears pertinent at this time to emphasize the well-known fact that the antibodies of certain animal species do not fix guinea pig complement with all antigens. In 1936, Horsfall and Goodner[19] reported that the capsular material of the pneumococcus fixed guinea pig complement with antibodies of the rabbit, guinea pig, rat, and sheep, but not with antibodies contained in sera from the horse, human, mouse, cat, dog, or goat. On the other hand, human complement is fixed by pneumococcus capsular material and human antibody.[15]

A similar phenomenon with Blastomyces antigens was reported in 1953.[29] The soluble polysaccharide substance washed off the surfaces of yeast-phase

cultures of *B. dermatitidis* fixed guinea pig complement with sera from hyperimmunized rabbits but not with sera from human patients with blastomycosis. On the other hand, the soluble protein material, liberated from within washed cells by sonic vibration, fixed guinea pig complement with both types of sera.

Such findings do not justify the conclusion that similar phenomena occur in other mycoses. It is emphasized that hyperimmune sera are most useful in detecting and characterizing fungus antigens but that tests with human sera from naturally-infected patients are required before such antigens can be considered of practical diagnostic value.

A protein-free complement-fixing antigen specific for *H. capsulatum*, precipitated from the supernates of yeast-phase cultures, was reported recently[56] but the antigen was tested only with hyperimmune rabbit sera which have the properties of fixing complement with polysaccharide antigens. The results suggest that studies of fractions isolated from other fungi by similar methods may reveal more specific antigenic characteristics, but it has to be kept in mind that such nonprotein fractions may be inactive as complement-fixing antigens in tests for human antibodies.

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It appears likely that the guinea pig complement-fixing component of Coccidioidin is different from that which elicits skin tests and yields a precipitate. Heated Coccidioidin does not fix complement with human serum but is potent as a skin-testing and precipitating antigen,[52] and the carbohydrate materials in Coccidioidin do not fix complement with human antibodies.[14,18]

Precipitin Tests.—In coccidioidomycosis, Smith and his associates[52] recommend tube tests in which aliquots of undiluted serum (with Merthiolate 1:10,000) are mixed with equal amounts of undiluted and 1:10 and 1:40 dilutions of Coccidioidin. The tubes are examined over a 5-day period for precipitate formation.

In histoplasmosis, Salvin and Hottle[42] recommend a capillary tube procedure, employing the constant antibody-varying antigen system for titration. The tests are read after incubating for 2 hours at 37° C. and refrigerating for 36 hours.

That the antigen-dilution procedure may not be applicable to estimations of antibody titer is suggested by studies of rabbit precipitins which indicated that the "titers" obtained actually represent measures of antigen and have no relationshlps to the antibody content of a serum.[6,27] Until similar investigations have been made with human serum it appears unwise to attribute significance to titers obtained by dilution procedures, and we concur with Smith's[52] practice of reporting precipitins as positive or negative regardless of the antigen dilution which yields a precipitate.

Practical Application of Immunologic Tests

The tests of choice and the interpretation of results are dependent upon the specific objective for which the test is performed.

1. *Determinimg Epidemiologic Patterns.*—Skin tests performed with well-standardized Coccidioidin or Histoplasmin are of unquestionable value in surveys designed to determine the geographic extent and other immunologic characteristics of Coccidioides and Histoplasma infections. Although either antigen may give a false positive test in an individual who is hypersensitive to some other

fungus, or possibly bacterium, such findings do not invalidate the use of either material as an epidemiologic survey tool. Negative findings in large groups of apparently normal individuals certainly can be interpreted as indicating the absence of infection. Results of the numerous Histoplasmin skin test surveys, which have been carried out in many areas of the world, have been summarized recently.[9]

Skin test surveys with Blastomycin have given negative results. Subclinical infections with *B. dermatitidis* apparently are rare or, if frequent, patients infected with Blastomyces do not become markedly hypersensitive to the fungus. In a skin test survey on 972 patients in a North Carolina state hospital for mental patients, only 15 (1.5 per cent) reacted to Blastomycin and 13 of these 15 reactors reacted to Histoplasmin.[28]

Skin tests for candidiasis are practically valueless for survey purposes since *C. albicans* is a most common endogenous infection. Positive tests with "Candidin" were obtained on 884 of 1,000 patients tested in Panama[59] and on 35 of 100 children tested from the Pittsburgh district with "Moniliin."[4]

2. *Finding Cases.*—In case finding it is believed that skin tests and all serologic tests available to the investigator should be used, including serologic tests with those antigens which have not been evaluated fully. Cases of early coccidioidomycosis would be missed unless both precipitin and complement fixation tests had been performed. Furcolow and Grayston[12] in their study of epidemics of histoplasmosis relied on 3 types of serologic tests—i.e., complement fixation tests with both Histoplasmin and yeast-phase antigens and precipitin tests. We are in agreement with the statement by Silverman and associates[51] that "the presence of a positive complement fixation test at levels considered critical by the specific laboratory where the tests are performed warrants further clinical and cultural investigation."

The procedures recommended above cannot be relied upon to pick up all cases of infection since some infected people may not develop antibodies, and in others, antibodies may be absent at the time blood is drawn. Clinical and mycologic studies therefore should be extended to all individuals exposed in common source epidemic situations even though the serologic findings are negative.

3. *Confirming Suspected Cases by Laboratory Examination.*—A diagnosis of mycotic disease can be established only after careful clinical and mycologic studies. Hypersensitivity and serologic tests must be considered solely as accessory clinical findings and are meaningless unless interpreted in terms of the specific illness of each individual patient. Skin tests with the homologous fungus antigen may be negative, and serologic tests may also be negative in proved cases of mycotic infection.

A positive serologic test with a single fungus antigen may be misleading since heterologous antigens may give higher titers than are obtained with an antigen prepared from the fungus causing the infection. A positive complement fixation test with Coccidioidin or with Blastomyces yeast-phase antigen, in any serum dilution, strongly suggests the presence of active infection with Coccidioides or Blastomyces if tests with one of the Histoplasma antigens are negative. On the other hand, tests with the more sensitive Histoplasma antigens may

be positive in heterologous fungus infections. In a series of tests on 63 sera from patients with proved blastomycosis 7 reacted negatively with the sonic-treated Blastomyces antigen, but fixed complement with a similarly prepared Histoplasma antigen.[29] A positive Histoplasmin collodion agglutination test or a positive low-titered complement fixation reaction with a yeast-phase Histoplasma is interpreted by Campbell and Binkley[1] as indicating only that the infection probably is mycotic in origin. It is most difficult to attach significance to titers obtained in serologic tests which utilize different Histoplasma antigens. Sera from apparently healthy Histoplasmin-sensitive individuals occasionally give complement fixation titers as high as 1:40 with the ground yeast-phase antigen, yet the same antigen may give negative results in sera from patients with chronic, proved histoplasmosis.[2] Repeated Histoplasmin skin tests in Histoplasmin-sensitive individuals also may stimulate antibody formation to the extent that titers as high as 1:40 are obtained by the collodion agglutination test[39,46] and by complement fixation tests with yeast-phase or Histoplasmin antigens.[46]

4. *Evaluating the Prognosis.*—In Coccidioidomycosis, a Coccidioidin complement fixation titer of 1:16 or above suggests that the infectious process is disseminating[52,53] and a titer of this magnitude also is indicative of a poor prognosis in blastomycosis.[26] The relationship between prognosis and antibody titer in histoplasmosis is not too clear since relatively few cases of progressive disease have been studied with a single type of Histoplasma antigen. The data obtained so far suggest that the situation in histoplasmosis is the reverse of that pertaining in blastomycosis or coccidioidomycosis in that lower titers are found in the chronic and more severe forms of the infection.[1,13,24]

A negative or a weakly positive skin test also is associated with the more serious forms of coccidioidomycosis,[52,53] blastomycosis,[26,55] and histoplasmosis.[60]

5. *Planning Therapy.*—The successful treatment of blastomycosis with the stilbamidines has minimized the importance of planning a therapeutic regime based on immunologic findings, as previously recommended.[30,34] However, occasionally a patient fails to respond to the drug but improves after receiving appropriate "immunologic" therapy.[23] The spectacular success of stilbamidine in blastomycosis has resulted in an increasing number of published case reports but, unfortunately, few reports contain immunologic data. Although it is recognized that such data are not vital to the modern management of the patient, such information is of the greatest importance in developing concepts concerning the patterns of immunologic responses in chronic infectious diseases.

Successful results obtained by vaccine desensitization or serum therapy in immunologically odd cases of pulmonary moniliasis have been reported.[16,26]

Discussion

Systemic fungus infections differ from those caused by bacteria and viruses in certain immunologic respects. In the latter types of infection it is customary to interpret the results of a serologic test as if a single antigen-antibody system were involved, although it is the general consensus that the serum from an infected patient probably contains variable quantities of specifically different antibodies. However, in diagnostic serologic tests for infections caused by most

bacteria and viruses, it is neither practical nor necessary to distinguish between the various antibodies which are produced. A positive agglutination test for brucellosis or for tularemia is a measure of the antibodies formed in response to the antigens located on the surface of the organism. Antibodies formed against internally situated antigens are not measurable by the agglutination procedure and little additional information of practical importance would be gained by testing for them. Furthermore, antibodies against surface antigens presumably are the most important defensive antibodies since the sensitization of surface antigens is a prerequisite for effective phagocytosis.

The recognition that acute primary pulmonary infections can be produced by *C. immitis* and *H. capsulatum* has changed many of our concepts concerning mycotic infections, and the serologic responses in such acute phases probably are similar to those which develop in bacterial infections. Tests for antifungal antibodies are complicated by the fact that simple agglutination tests are not practical. Most fungi are quite large and it is difficult to obtain suitable stable antigen suspensions; this necessitates recourse to complement fixation and precipitin tests with the various complex filtrate antigens and yeast-phase suspensions.

In the chronic and disseminating phases of mycotic infections I believe that it is expecially important to distinguish between the antibodies produced in response to the variously located antigens. The high titers of complement-fixing antibodies which are found in disseminating cases of coccidioidomycosis and in severe systemic blastomycosis suggest that these antibodies are not important as a defensive mechanism. In blastomycosis it has been demonstrated that the soluble antigen which fixes complement with human antibodies probably is located inside of the cell[29] although some antigen must be present on the exterior surfaces of the intact cells since unwashed cell suspensions fix complement with human sera.[25] The antigen formed within the cell may diffuse through and become attached to the cell wall, or the antigen actually may be an integral part of the cell wall itself. Of more importance is the fact that the fungus forms a very soluble nonprotein antigen which can be found in supernates of yeast-phase suspensions[29] or in the supernates of yeast-phase broth cultures[7] of Blastomyces and in the supernates of yeast-phase broth cultures of Histoplasma.[56] I believe that such soluble materials are entirely different from the complement-fixing antigens and must be taken into consideration in order to understand the immunologic aspects of the disease process.

Caution should be exerted in attributing significance to the serologic responses of hyperimmunized animals in terms of their application to problems concerned with the serology of human infections. As mentioned above, certain types of antigens fix guinea pig complement with rabbit but not with human antibodies. It is emphasized also that the immunologic responses of an infected host may be quite different from those of a hyperimmune animal. In the later situation, killed organisms usually are injected by the unnatural intravenous route, and bloods are taken at optimal time intervals. In infected animals and individuals the organism is living and multiplying and is continuously excreting antigenic materials and other metabolic products. In severe infections, soluble antigens may be diffused into the tissues of the host and combine with preformed

antibodies to the extent that serologic titers may be lowered. Such an interpretation is suggested by the observation in blastomycosis that antibodies to the soluble antigen, as measured by hemagglutination tests, are found in lower titers in patients with poor prognoses although the complement fixation titers are high.[29] Serologic studies in coccidioidomycosis suggest that a similar phenomenon may occur, in that precipitins may be negative in patients with serious disseminating disease. In all three diseases (coccidioidomycosis, blastomycosis, and histoplasmosis) patients with poor prognoses show marked reductions in their skin test responses, suggesting the interpretation that the inhibition of hypersensitivity may be due to an excess of soluble antigen in the tissues.

The above hypothesis was advanced in 1941 as a possible explanation of some unusual immunologic findings in a patient with blastomycosis[30] who reacted negatively to the Blastomyces skin-testing material and had a negative complement fixation test. However, the patient gave an immediate "wheal" reaction to the intracutaneous injection of an anti-Blastomyces hyperimmune rabbit serum. After treatment with several small doses of hyperimmune serum the patient not only recovered but developed a positive skin test to the vaccine and a negative skin test to the antiserum. A similar experience was encountered in a patient with severe candidiasis who reacted negatively to a *C. albicans* vaccine but gave an immediate response to hyperimmune anti-*C. albicans* serum.[16] That an excess of circulating antigen may occur in mycotic infections was demonstrated beautifully by Neill, Sugg, and McCauley,[35] who proved the presence of Cryptococcus capsular antigens in the blood, urine, and spinal fluid of a patient dying of cryptococcosis.

In histoplasmosis it is most difficult to interpret the results of serologic tests as performed with different types of antigen, especially when there are such marked differences in the antigen-producing capacities of different strains.[50] In tests with yeast-phase and filtrate antigens, both prepared from a single strain of *H. capsulatum* which was known to produce a good complement-fixing Histoplasmin, it was observed that the Histoplasmin gave markedly higher complement fixation titers than the yeast-phase antigen when tested with sera from culturally proved cases of histoplasmosis. In sera from hyperimmunized rabbits the same yeast-phase antigen gave slightly higher complement fixation titers than did the Histoplasmin. However, a later comparison of yeast-phase antigens produced from 11 different strains of *H. capsulatum* showed that the strain used in the previous study, which had been selected because of its known ability to produce a good Histoplasmin complement-fixing antigen, was one of the poorest producers of yeast-phase antigen.[49] Consequently, the relatively poor showing of the yeast-phase antigen recorded in the previous study[48] probably was due to strain differences.

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A BIBLIOGRAPHY OF INTERNAL MEDICINE: GONORRHEA AND GONOCOCCAL INFECTION

ARTHUR L. BLOOMFIELD

SAN FRANCISCO, CALIF.

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THE early literature on gonorrhea is concerned largely with differentiation of the disease from syphilis, and the reader is referred to the section on syphilis of this bibliography for pertinent references. Innumerable books are available on the subject of gonorrhea of which that by Ernest Finger (*Die Blenorrhöe der Sexualorgane und ihre Complicationen*, Leipzig und Wien, 1888, Frantz Deuticke) is the classical text which ran through many editions and translations. Gonorrhea is usually discussed in the general textbooks on venereal and genitourinary diseases, and many important articles lie buried in journals on the eye and other special branches. Much, of course, is to be found in the gynecological literature from the time of Bumm (Ref. 10) on. Among modern texts one should mention the large well-documented treatise by Charles C. Norris (*Gonorrhoea in Women*, Philadelphia and London, 1913, W. B. Saunders Company) and the standard text by P. S. Pelouze (*Gonorrhoea in the Male and Female*, ed. 3, Philadelphia and London, 1939, W. B. Saunders Company) which ran through many editions.

We have not attempted to follow the bibliography through all the ramifications of the special domains in which the gonococcus may cause disease; we have had in mind that this bibliography is primarily for medical students and physicians with general interests.

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GONORRHEA AND GONOCOCCAL INFECTION

Clinical—Refs. 1, 2, 10.
Differentiation from syphilis—Refs. 1, 2.
General—Refs. 1, 2, 10.
Gonococcal arthritis—Ref. 13.
Gonococcal endocarditis—Ref. 15.
Gonococcal infection in females—Refs. 9, 10, 11.
Gonococcal lesions of the skin—Ref. 14.
Gonococcal ophthalmia—Ref. 5.
Gonococcus—Refs. 3, 4, 6, 7, 8, 10, 12.
Treatment with antibiotics—Ref. 16.

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1. Bell, Benjamin: A Treatise on Gonorrhoea Virulenta and Lues Venerea, Edinburgh, 1793, James Watson and Co., 2 Vols.

Bell was the first clearly to differentiate gonorrhea from syphilis and to insist that it was an independent disease. After a general introduction he devotes a full volume to the discussion of the "gonorrhoea virulenta." "Every discharge of matter from the urethra, excited by impure coition, is termed Gonorrhoea Virulenta." There follow chapters on clinical features of the disease going into minutest details of symptoms and course. "The duration of gonorrhoea has always been a matter of much uncertainty." He points out that with simple urethritis "the disease will not commonly endure a fortnight . . . but, wherever the lower parts of the urethra are affected, particularly when the prostate gland and other parts about the neck of the bladder are diseased, the running, in almost every instance proves obstinate" (p. 67). Bell, along with others, questioned the value of the medicines in vogue: "A low diet, mercury, and evacuants of different kinds, did much harm, as we have already observed, by inducing such a degree of debility and relaxation as materially affected the constitution" (p. 74). The pros and cons of local injections and the use of sounds are discussed. The first part of the book ends with an excellent recapitulation: "It appears that Gonorrhoea is a local disease, proceeding from a specific contagion, and not necessarily connected with any other . . . that the discharge of matter which takes place is not the effect of ulceration, but proceeds from an inflamed state of the urethra and contiguous parts . . . that the disease is always formidable in proportion to the depth of parts that are affected . . . that in the cure of Gonorrhoea no advantage is derived from mercury, or any remedy acting altogether upon the constitution [in contrast to syphilis] . . . that where the membrane of the urethra is alone affected, no remedy proves so successful as astringent injections . . . but that they are never employed but with much risk of doing harm where the inflammation has reached Cowper's glands, to the prostate gland or to the bladder . . . that when the bladder is affected, opium or hyoscyamus are in like manner to be used to relieve pain" (p. 168 et seq.). Bell described all sorts of local complications of gonorrhea but was entirely unaware of distant metastases such as arthritis, ophthalmia, etc. The book is nonetheless a masterpiece, based as it is on accurate observation of clinical cases.

2. Ricord, Ph.: Traité pratique des maladies vénériennes, Paris, 1838, De Just Rouvier et E. le Bouvier.

The book by Ricord, the great French expert on venereal disease, deals with both syphilis and gonorrhea although he believed them to be different diseases and proved his point by a great many intradermal inoculations of gonorrheal pus into volunteers without result, whereas if the material were syphilitic, chancres would be expected to appear. Ricord went further than Bell in recognizing some distant complications such as gonococcal ophthalmia. "Its development must be attributed to the direct application of the gonorrheal matter to the conjunctiva . . . the first thing to be urged is speed and energy. . . . Hesitation and uncertainty are often followed by loss of the eyes . . . one must always insist on applications of silver nitrate" (p. 761). The method of application is described in detail. Ricord does not, however, mention the common occurrence of gonococcal arthritis.

3. Neisser, Albert: Ueber eine der Gonorrhoe eigentümliche Micrococcusform, Centralbl. f. d. med. Wissenschaften *17:* 497, 1879.

The idea of a bacterial cause of gonorrhea was in the air. Thus Lebert (*in* H. v. Ziemssen: *Handbuch der Speciellen Pathologie und Therapie*, Leipsic, 1875, F. C. W. Vogel, Vol. 9, part II, p. 295), four years before Neisser's discovery, says, "It is therefore highly probable that gonorrheal pus contains a contagious principle, which frankly the microscope and chemical analysis have so far not found. . . . One can suppose that germs develop in the depths of the urethral mucosa." Various workers were searching for such a cause at the time. In this modest and conservative report Neisser announced the discovery of the gonococcus. The organisms were seen in pus from gonococcal urethritis spread thin on slides and stained with methyl-violet. The intracellular predominance, the biscuit-shaped pairs of recently divided cocci are well described. These bacteria were found in 35 cases of gonorrhea varying in duration—three days to thirteen weeks—but not in a chronic case of one and one-half years. The gonococcus seemed to be the only organism present. Neisser isolated it from nine purulent urethritides in women, and from two patients with gonorrheal conjunctivitis. Neisser reserves final judgment as to the significance of the organisms pending cultural and inoculation experiments.

Neisser's work was soon confirmed in other countries as for example by P. Weiss (Thèse de Nancy, Ire série, No. 119, 1880) an abstract of whose work is reported in Ann. de dermat. et de syph. (Le microbe du pus blennorhagique) *2:* 189, 1881. Weiss found Neisser's coccus in the gonorrheal discharges of both men and women and apparently was the first to advise the use of potassium permanganate, 0.25 per 1,000, irrigations later much employed. O. Haab (Kleinere ophthalmologische mittheilungen. II. Parasitäre Augenkrankheiten, Cor.-Bl. f. schweitz Aerzte *11:* 79, 1881) early confirmed the finding of typical cocci in the discharge of conjunctivitis of the newborn and in gonorrheal ophthalmia. He was unable, however, to grow the organisms in culture.

4. Leistikow: Ueber Bacterien bei den venerischen Krankheiten, Charité-Annalen *7:* 750, 1880.

Leistikow reports confirmation of Neisser's work. He also undertook culture studies with Löffler and after many failures the "cultures finally throve in a blood serum gelatine at body temperature." They tried many inoculation experiments in rabbits, dogs, rats, mice, guinea pigs, and horses with failure in all to produce disease. The results in a monkey were uncertain. Leistikow was probably the first to report successful cultivation of the gonococcus. Chemotherapy was already under way and he discussed the relative merits of injections of bichloride, zinc solutions, tannic acid, lead acetate, silver nitrate, and carbolic acid. The results were hard to evaluate since gonorrheal discharges ceased of themselves with time.

5. Credé [C.]: Die Verhütung der Augenentzündung der Neugeborenen, Arch. f. Gyn. *17:* 50, 1881.

Starting from the assumption that conjunctivitis of the newborn was acquired from gonococcal infection of the mother during the baby's passage through the birth canal, Credé began prophylactic treatment of all newborn babies in the Leipsic clinic. He first used various solutions, but "from 1 June 1880 all eyes without exception were disinfected directly after birth . . . with a single drop of nitrate of silver (1:50)." At the same time douching of the mother was discontinued. "All the children so treated have escaped eye inflammation even of slight grade." Slight hyperemia and increased secretion might be present in the first twenty-four hours but no harm was ever done to the eyes. "The main point is in the experience that not disinfection of the vagina but only of the eye achieves the goal." The incidence of conjuctivitis fell in the clinic from 13.6 in 1874 to 0.5 in 1880. In a further communication (Ibid. *18:* 367) Credé reported 400 new cases in addition to 200 previously recorded. The babies' eyes were wiped with a cloth dipped in plain water and then "one drop of 2 per cent solution of silver nitrate was instilled. No child so treated developed ophthalmia neonatorum in the first 7 days of life." Credé's method was soon generally in use the world over and was relied upon for many years. His work was summarized in a book (*Die Verhütung der Augenentzündung der Neugeborenen*, Berlin, 1884, A. Hirschwald).

Penicillin has recently been tried in place of silver nitrate as a prophylactic against gonococcal conjunctivitis. The relative merits of the two are analyzed by H. C. Franklin (Prophylaxis Against Ophthalmia Neonatorum, Clinical Comparison of Penicillin and Silver Nitrate; Preliminary Report, J.A.M.A. *134:* 1230, 1947). He leans, on the whole, to penicillin, although the diminished frequency of maternal gonorrhea makes the problem much less urgent than in Credé's day.

6. Krause, Fedor: II. Die Micrococcen der Blennorrhoea neonatorum, Centralbl. f. prakt. Augenheilk. *6:* 134, 1882.

Krause succeeded in growing gonococci from blennorrhea neonatorum, using coagulated blood serum as a culture medium. He obtained diplococci in pure culture from 12 children. Inoculation of the conjunctivae of various

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small animals was unsuccessful. This work was done in Hirschberg's clinic, and Hirschberg himself is reported (Gesellschaft der Charité-Aerzte in Berlin, 16 Feb., 1882, Berl. klin. Wchnschr. *19:* 500, 1882) to have made inoculations with gonorrheal pus into the human eye, and to have invariably produced disease, although animal inoculation failed. At the same meeting early attempts at treatment of gonorrheal urethritis are reported. Lewin used bichloride of mercury injections (1: 20,000), Güterbock reported that irrigations with water were just as effective, and Leistikow found that patients recovered equally well without any irrigations.

7. Neisser, Albert: IV. Die Micrococcen der Gonorrhoe, Deutsche med. Wchnschr. *8:* 279, 1882.

Neisser gives a definitive review of the subject to date. He first makes it clear that the gonococcus is a specific organism and describes again its morphological features: biscuit-shaped diplococci, "adherent" to pus cells. "Gonococci are absolutely constant in every case of gonorrhea . . . and are not found in any other disease. . . . Furthermore the gonococci are the only organisms which are found in gonococcal pus." He now reports success in cultivation of gonococci on meat-extract-peptone gelatin. "As far as one can ever tell anything by microscopic examination, all the doctors who looked at the preparations say that the cultures are pure growths of gonococci (H. A. Cohnheim, Koch, Ehrlich)." Inoculation of humans with cultures failed, however, as well as animal inoculations. Neisser was unable to find gonococci in pus from gonococcal arthritis and epididymitis but he stressed the occurrence of the germs in ophthalmia.

However, not everyone accepted the gonococcus as the specific cause of the disease and various conflicting reports in the literature on specificity and pathogenicity of "gonococci" are reviewed by Bumm (Ref. 10).

8. Bockhart, Max: Beitrag zur Aetiologie und Pathologie des Harnröhrentrippers, Vrtljschr. f. Dermatol. u. Syph. *15:* 3, 1883.

Bockhart undertook to inoculate a normal male urethra with a pure culture of gonococci which he received from Dr. Fehleisen. The subject was a man with advanced dementia paralytica. Four days later there was a full-blown urethritis with countless gonococci in the secretion. A few days later the patient died and at autopsy the sections of the urethra showed gonococci. Here then seemed to be undoubted proof that the gonococcus was the pathogenic agent in urethritis. Bockhart builds up a theory of pathogenesis of gonorrhea on the basis of lymphatic invasion. The distant complications—endocarditis, arthritis—come, he feels, from extension of infection into the blood stream.

A full discussion of the subject of experimental gonorrhea with extensive bibliography is given by J. H. Hill (Experimental Infection With N. Gonorrhoea. I. Human Infection, Am. J. Syph., Gonor. & Ven. Dis. *27:* 733, 1943; Idem. II. Animal Inoculations, Ibid. *28:* 334, 471, 1943). J. F. Mahoney, C. I. Van Slyke, J. C. Cutler, and H. L. Blum (Experimental Gonococcic Urethritis in Human Volunteers, Ibid. *30:* 1, 1946) deal further with the same problem.

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9. Arning, E.: Ueber das Vorkommen von Gonococcen bei Bartolinitis. Vrtljschr. f. Dermatol. u. Syph. *15:* 371, 1883.

Arning found gonococci regularly in acute and chronic inflammation of Bartholin's glands in women. At about the same time E. Bumm (Beitrag zur Kentniss der Gonorrhoe der weiblichen Genitalien, Arch. f. Gyn. *23:* 328, 1884), the gynecologist, made a systematic study of gonococcal infection in women and pointed out the "most frequent site of gonorrhea is the mucosa of the cervix uteri." But to confuse matters Bumm found organisms resembling gonococci in the genitals of women "where the possibility of gonorrhea was absolutely excluded." These cocci were biscuit-shaped diplo-forms and were readily cultivable on artificial media, where they grew out as milk-white plaques, which produced no lesion when inoculated on mucous membranes. One suspects that these bacteria were members of the *N. catarrhalis* group. They were also found in ulcerative processes in the mouth and in sputum.

10. Bumm, Ernst: Der Micro-Organismus der Gonorrhoischen Schleimhaut-Erkrankungen, "Gonococcus-Neisser," Wiesbaden, 1885, J. F. Bergmann.

This little book, which is a definite landmark in the subject, opens with a critical review, to date, of the literature on the gonococcus. There follows a description of the morphology and staining, and a discussion of numerous biscuit-shaped diplococci other than gonococci—a lemon-yellow diplococcus, not pathogenic, a milk-white diplococcus, not pathogenic, a yellow-white diplococcus, pathogenic, a pink diplococcus, not pathogenic. All these need to be differentiated from the gonococcus. He lists the "pathologic secretions" in which the gonococci occur: male and female urethritis, bladder and kidney, periurethral abscesses, knee joint, conjunctiva, rectum, body and cervix of the uterus, Bartholin's glands. Bumm summarizes the position of the gonococcus in diagnosis: "The presence of Neisser's gonococcus in the secretions proves the infectious origin of the disease of the mucous membrane and conversely gonococcus-free secretion has no virulent characteristics." Bumm gradually assembled a considerable amount of pathological tissue from which he was able to build up the histological appearance of gonococcal infection day by day until the thirty-third day—a fundamental piece of work. He next summarizes the literature on attempts to cultivate the gonococcus on artificial media and describes his own successful attempts, pointing out the difficulties and the ease with which confusion could arise with other diplococci which were readily cultivable. He feels that Neisser did not grow genuine gonococci, whereas Fehleisen probably did and Leistikow definitely did. Bumm inoculated the urethra of a healthy woman and produced gonococcal urethritis, once more proving the pathogenic specificity of the organism.

Bumm's summary brings out very well the difficulties of the subject at the time; principally the confusion of gonococci with other diplococci in smear and in culture. He also speculates about distant lesions, as to whether they are due to spread of gonococci through the body or whether they are "sympathetic" or due to an absorbed toxin, questions which vexed everyone at the time. However his monograph was definitive to date and left relatively little of importance untouched.

11. Fraenkel, Eugen: Bericht über eine bei Kindern beobachtate Endemie infectiöser Colpitis, Virchow's Arch. f. path. Anat. *99:* 251, 1885.

The syndrome of vulvovaginitis in little girls was recognized in the early part of the nineteenth century as pointed out in the excellent reviews of the literature of Cahen-Broch (Die urogenital-Blenorrhoe der kleinen Mädchen, Jahrb. f. Kinderheilk. *34:* 369, 1892) and of A. Epstein (Ueber Vulvovaginitis gonorrhoica bei kleinen Mädchen, Arch. f. Dermat. u. Syph. *23:* 3, 1891). The occurrence in little girls, the relative immunity of boys and of girls after menstruation starts, the usual nonvenereal nature of the spread of the disease, the association of the child with a mother who has gonorrhea, the occurrence in institutions where many children are in close contact and where a common bathtub and towels are used, are all stressed. R. Pott (Die specifische Vulvo-Vaginitis im Kindesalter und ihre Behandlung, Jahrb. f. Kinderheilk. *19:* 71, 1883) was among the first to insist that this disease was "the expression of a specific communicable disease of the mucous membrane," and to point out how widespread it was in the general population. These writers give detailed descriptions of the findings and clinical course of the condition, and the chronic character and great resistance to disinfectants and astringents are noted by everyone.

Fraenkel, however, was the first to definitively establish the gonococcus as cause of the disease. He studied the discharges and "the finding was very striking, since there was success in demonstrating in every preparation the presence of a micro-organism which differed in no respect from the gonococcus of Neisser."

The large modern literature on the subject is reviewed by R. A. Benson and A. Steer (Vaginitis of Children, Am. J. Dis. Child. *53:* 806, 1937). Recently, A. Cohn, A. Steer, and E. L. Adler (Gonococal Vaginitis, Ven. Dis. Inform. *21:* 208, 1940) could only prove that 23.3 per cent of cases of juvenile vaginitis were definitely gonococcal, and they place more emphasis than most observers on actual sexual contact. However, both sulfanilamide and sulfapyridine produced rapid cures. Like all other manifestations of the gonococcus, vaginitis responds promptly to penicillin (B. G. Clarke, and H. H. Eisenberg: Gonococcic Vaginitis in Children Treated With Single Injection of Penicillin in Beeswax and Peanut Oil; Report of Twenty Cases, Am. J. Dis. Child. *74:* 707, 1947).

12. Wertheim, Ernst: Reinzüchtung des Gonococcus Neisser mittels des Plattenverfahrens, Deutsche med. Wchnschr. *17:* 1351, 1891.

Wertheim modified previous cultural procedures so that he readily grew gonococci in plates of serum-peptone agar. He inoculated the urethras of five men with general paresis and in every case produced typical gonorrhea from which the gonococci were again recovered. Wertheim considers that his experiments give final and indisputable proof that the coccus of Neisser is actually the cause of gonorrhea. The observation has been often repeated by various observers.

Modern work on the complexity of suitable media for growth of the gonococcus is summarized in the article by J. F. Mahoney and J. D. Thayer *in* R. J.

Dubos: *Bacterial and Mycotic Infections of Man* (ed. 2, Philadelphia, 1952, J. B. Lippincott Co., p. 565). As methods have been improved cultures have been found to have as valuable a place in diagnosis as direct smears (see Carpenter, C. M. et al.: Evaluation of Twelve Media for the Isolation of the Gonococcus, Am. J. Syph., Gonor. & Ven. Dis. *33:* 164, 1949, and Thayer, J. D., Schubert, J. H., and Bucca, M. A.: The Evaluation of Culture Mediums for the Routine Isolation of the Gonococcus, J. Ven. Dis. Inform. *28:* 37, 1947).

13. Höck, Heinrich: Ein Beitrag zur Arthritis blennorrhoica, Wien. klin. Wchnschr. *6:* 736, 1893.

The concept of gonococcal arthritis seems to have grown slowly so that Brandes of Copenhagen (Du rhumatisme blennorrhagique, Arch. gén. de méd. *4:* 257, 1854) could open his paper with the question "does blenorrhagic rheumatism really exist?" Apparently some of the alleged cases were only instances of vague aches and pains, or they may have been some other variety of arthritis. Brandes reports several cases of gonorrhea associated with arthritis but as there was no bacteriological confirmation they are difficult to interpret. He points out that this form of arthritis is much more common in men than in women and that it often accompanies ophthalmia. "The complication of cardiac lesions is rare, very rare, but it is not impossible." The knee is the joint most often affected, although the trouble migrates from joint to joint. Ankyloses of large joints rarely occur. G. Bücker (Ueber Polyarthritis gonorrhoica, Inaug. Diss., Berlin, 1880) reviews the whole subject and reports two cases in which pus was withdrawn from joints, but apparently no search was made for Neisser's coccus.

A step forward was made by L. M. Petrone (Sulla natura parasitaria dell'-artrite blenorrhagica, Riv. clin. di Bologna *3:* 94, 1883, which is abstracted in Centralbl. f. Chir. *10:* 586, 1883) who found in smears from the joint exudate of two cases of urethritis and arthritis bacteria which corresponded in every respect with Neisser's organisms. One does not know what to make of the statement that the cocci were seen also in the blood, presumably in smears. In the following year, F. Kammerer (Ueber gonorrhoische Gelenkentzündung, Centralbl. f. Chir. *11:* 49, 1884) also saw typical gonococci in the exudate of an inflamed knee joint, but neither of these observers brought the final proof of the existence of gonococcal infection of joints by cultivating the organism.

Höck seems to have been the first to isolate gonococci from a joint which was the seat of acute arthritis. Interestingly enough his patient was a baby with gonococcal ophthalmia; he reviews a number of other instances of arthritis following gonococcal ophthalmitis in which organisms were seen in smears but were not cultured. Neisser (Ueber die Züchtung der gonococcen bei einen Fall von Arthritis gonorrhoica, Deutsche med. Wchnschr. *20:* 160, 1894), shortly after, reported culture from the knee and finger of an adult with gonococcal arthritis, and finally Bordoni-Uffreduzzi (Ueber de Localization des Gonococcus im Inneren des Organismus [duch den Gonococcus herforgerufene Pleuritis und Arthritis], Ibid., p. 484) produced urethritis in a volunteer with a culture of gonococcus obtained from a case of arthritis. That the gonococcus could spread in the body and produce distant lesions now seemed understandable.

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C. S. Keefer and W. W. Spink (Gonococcic Arthritis: Pathogenesis, Mechanism of Recovery and Treatment, J.A.M.A. *109:* 1448, 1937) give a definitive modern discussion of the subject up to the antibiotic era. They point out certain associated lesions, such as metastatic catarrhal conjunctivitis, iridocyclitis, and keratodermia blenorrhagicum, and the occasional occurrence of bacteremia even without endocardial involvement. In another paper (Latent Gonorrhoea as a Cause of Acute Polyarticular Arthritis, J.A.M.A. *109:* 325, 1937) they emphasize the fact that gonococcal arthritis may occur when the primary urethral infection gives no overt manifestations.

The modern therapy of gonococcal arthritis begins with the use of artificial fever, the results of which are summarized by R. M. Stecher and W. M. Solomon (The Treatment of Gonorrhoeal Arthritis With Artificial Fever, Am. J. M. Sc. *192:* 497, 1939). Of a series of 50 cases, 26 patients were relieved of all joint symptoms, 11 received benefit, while 13 were not helped. Because of the strenuous and complicated character of fever therapy, it was soon replaced by chemotherapy when the sulfonamides were found effective. C. S. Keefer and L. A. Rantz (Sulphanilamide in the Treatment of Gonococcal Arthritis, Am. J. M. Sc. *197:* 168, 1939) were the first to report a careful study. They found that in adequate dosage the drug diffused into the synovial fluid, which was sterilized in several days. The "results are encouraging and all patients with gonococcal arthritis should be treated intensively with the drug." J. H. Linner (Suppurative Myositis and Purulent Arthritis Complicating Acute Gonorrhoea, J.A.M.A. *123:* 757, 1943) was the first to report a case treated with penicillin. N. Spitzer and O. Steinbrocker (The Treatment of Gonococcal Arthritis With Penicillin, Am. J. M. Sc. *218:* 138, 1949) later reviewed the subjects and found that among 28 cases "23 were regarded as cured or greatly improved, 5 as failures."

14. Vidal, Emile: Éruption généralisée et symétrique de croûtes cornées avec chute des ongles, d'origine blenorrhagique, coïncidante avec une polyarthrite de même nature. Récedive à la suite d'une nouvelle blenorrhagie, deux ans après la guérison de la première maladie, Ann. de Dermat. et de Syph. *4:* 3, 1893.

Vidal appears to have been the first to describe thoroughly the skin lesions of gonorrhea and to note their association with arthritis. "The recurrence of these lesions the pathogenesis of which was in doubt in 1890 shows that they are a direct manifestation of the gonorrheal infection, just as arthritis is." J. G. Downing (Keratoderma Blenorrhagicum, J.A.M.A. *102:* 829, 1934) reviews the literature with a case report of his own, as do W. W. Spink and C. S. Keefer (The Renal and Dermatologic Complications of Gonococcal Infection, New England J. Med. *217:* 241, 1937). The first American case was that of F. E. Simpson (Kératodermie blenorrhagique, J.A.M.A. *59:* 607, 1912) whose paper is especially useful since it contains abstracts of the previously reported cases. A. W. Frerich, S. Schwartz, and O. Steinbrocker (Penicillin in the Treatment of Keratosis Blenorrhagica With Polyarthritis, Arch. Int. Med. *79:* 239, 1947) treated three cases with penicillin, followed by brilliant cures in two of them.

15. Thayer, William Sidney, and Blumer, George: Ulcerative Endocarditis due to the Gonococcus; Gonorrhoeal Septicemia, Bull. Johns Hopkins Hosp. *7:* 57, 1896.

J. Marty (De l'endocardite blenorrhagique, Arch. gén. de méd. *28:* 660, 1876) gives brief excerpts of several cases from the literature and reports two of his own in which an attack of gonorrhea was followed by arthritis and signs of endocarditis. He gives an excellent discussion of the whole subject, but as his own patients did not die and as there was no bacteriological confirmation, it remains uncertain whether these were really cases of gonococcal endocarditis. Previously Lacassagne (Des complications cardiaques de la blenorrhagie, Arch. gén. de méd. *19:* 15, 1872) reviewed the subject of serous membrane involvement with gonorrhea and he reports a number of cases of pericarditis, including one of his own which he regards as of gonococcal origin. Thayer and Blumer review the reports of several recent cases of endocarditis in which gonococci were presumably seen in smears from the heart valves at autopsy but the final proof of cultures was not obtained. Their own patient ran the typical course of ulcerative endocarditis. At autopsy there were vegetations of the mitral valve containing what were morphologically typical gonococci. The organisms were recovered in blood culture during life and were grown from the valve after death. Here then was the first proved case of gonococcal sepsis with ulcerative endocarditis. Modern statistics on this lesion may be found in Thayer's monograph (Studies on Bacterial (Infective) Endocarditis, Johns Hopkins Hosp. Reps. *22:* Fasc. I, 1926). W. S. Thayer and J. W. Lazear (A Second Case of Gonococcal Septicemia and Ulcerative Endocarditis With Observations Upon the Cardiac Complications of Gonorrhoea, J. Exper. Med. *4:* 81, 1899) soon reported a second case of gonococcal bacteremia with extensive vegetations on the tricuspid valve and multiple purulent lesions. They review the literature and point out that there are five cases (including their own two) in which the gonococcal nature of the infection is definitely proved. But they go further and emphasize that the gonococcus may on occasion act exactly like the pyogenic cocci and produce a general sepsis with multiple purulent lesions and bacteremia. "Grave myocardial changes, necrosis, purulent infiltration, embolic abscesses are common in severe gonococcal septicaemias . . . the diagnosis may in some cases, be made during life by cultures taken from the circulating blood." The article is a classic. Thayer later (On the Cardiac Complications of Gonorrhoea, Bull. Johns Hopkins Hosp. *33:* 361, 1922) again wrote on the subject.

One wonders what effect modern drugs and antibiotics would have on this serious disease. E. S. Orgain and M. A. Poston (Gonococcal Endocarditis, With Recovery After Sulfapyridine, New England J. Med. *221:* 167, 1939) report a case with recovery after treatment with sulfapyridine. P. H. Futcher and V. C. Scott (Four Cases of Gonococcal Endocarditis Treated With Sulfanilamide, With Recovery of One, Bull. Johns Hopkins Hosp. *65:* 377, 1939), using strict criteria, had one recovery after transfusion and sulfanilamide, whereas three other patients treated with sulfanilamide all died. To further complicate the interpretation, spontaneous recovery or recovery following transfusion alone

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has been reported fairly often. Futcher and Scott review the literature on "spontaneous" recovery from gonococcal endocarditis and bacteremia.

16. Herrell, W. E., Cook, E. N., and Thompson, L.: Use of Penicillin in Sulfonamide Resistant Gonorrheal Infections, J.A.M.A. *122:* 289, 1943.

E. P. Abraham et al. (Further Observations on Penicillin, Lancet *2:* 177, 1941) had shown that the gonococcus was highly sensitive to penicillin in the test tube; Herrell and his associates confirmed this observation and seem to have been the first to use the material in clinical gonorrhea. Because of very limited amounts of penicillin available they treated only sulfonamide-resistant cases. "The complete absence of toxicity following the intravenous administration of pyrogen free penicillin, the lack of any discomfort to the patient and the rather rapid disappearance of clinical symptoms have been observed in three cases of sulfonamide resistant gonorrheal infections. . . . In all the cases reported, in addition to the clinical response noted, negative bacterial cultures were obtained some time between seventeen and forty-eight hours after the institution of penicillin therapy." A little later J. F. Mahoney, C. Ferguson, M. Buchholtz, and C. J. Van Slyke (The Use of Penicillin Sodium in the Treatment of Sulfonamide-Resistant Gonorrhea in Men, Am. J. Syph., Gonor. & Ven. Dis. *27:* 525, 1943) reported cure of seventy-two of seventy-five cases of gonorrhea with a total of only 160,000 units of penicillin over a treatment period of forty-five hours. Since then, the supremacy of penicillin in treatment of gonorrhea has been universally confirmed, as, for example, by T. H. Sternberg and T. B. Turner (The Treatment of Sulfonamide Resistant Gonorrhea With Penicillin Sodium, J.A.M.A. *126:* 157, 1944). C. P. Miller, W. W. Scott, and V. Moeller (Studies on the Action of Penicillin. I. The Rapidity of Its Therapeutic Effect on Gonococci Urethritis, J.A.M.A. *125:* 607, 1944) found that intramuscular doses of 50,000 to 100,000 units of penicillin were followed by rapid disappearance of gonococci from cases of urethritis.

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Editorial

THE JOURNAL AS A STIMULUS TO RESEARCH

In the Announcement in the first issue of the JOURNAL OF CHRONIC DISEASES it was underscored that "In recognition of the urgent fact that chronic illness affects persons of all ages, this Journal is more interested in its beginnings than in its endings." It was stated also that "The Editorial Board has wished to avoid too rigid a definition of the content of the JOURNAL, preferring to be guided by the experience of the next few years, and by the desires of its readers. The Editor and Associate Editor will welcome comments and suggestions." We wish to respond to this invitation and to enlarge upon the implications of the major interest of the JOURNAL as stated above.

Full understanding of the many and complex processes which have a bearing on the "beginnings" of chronic illness (as well as on its "endings") require careful examination and synthesis of the facts which emerge from all forms of observation and experimentation on problems of health. Therefore, to achieve its major stated aim, the JOURNAL should seek to provide a forum for the presentation, discussion, and synthesis of the facts and ideas obtained from research on chronic illness approached from three directions: (1) the laboratory, (2) the sick person, (3) the population in the community.

Laboratory research furnishes the means (a) to understand the basic biologic and physicochemical processes which take place in the living organism, and (b) to test in a simplified situation hypotheses on causation and treatment of disease. Clinical research provides knowledge of the characteristics of disease processes in the person and on the effectiveness of treatments. Population research is necessary (a) for the discovery of the factors within the family and within the physical and biologic environment which produce disease or supply fertile ground for it; (b) for knowledge of the impact of the disease on the individual, on his family, and on the community.

By providing a forum for the dissemination and synthesis of the findings of all three research approaches to the study of chronic illness, the JOURNAL contributes to advances in all three kinds of research. This is so, because progress in one depends on progress in the others. Laboratory research is limited in formulating the questions to be investigated until it has at hand some results of clinical and population research. Progress in clinical research is dependent upon knowledge regarding causation and therapy obtained through population and laboratory research. Similarly, population research cannot progress far without a precise characterization of the disease entity as observed at the bedside or as delineated by the findings in the laboratory.

The interdependence of the three research approaches implies that all three should be encouraged. The JOURNAL shares with many other journals the function of disseminating facts and ideas derived from laboratory and clinical research, but it is one of the few periodicals that seeks, publishes, and discusses the findings of population research on chronic illness. It is not often realized that investigators who conduct population research on chronic illness have few opportunities for publication of their findings. There are a number of persons and organizations throughout the country who are engaged in conducting studies on one or another segment of the population in the community but little information on the findings become available to the student of chronic illness. We believe that there are many potential investigators in schools of public health, in medical schools, in clinics and hospitals, in schools of social work, in schools of nursing, and departments of sociology, etc., who would be in a position to initiate and carry out such studies if properly stimulated. The JOURNAL can do much to encourage publication of findings and to stimulate the initiation of population research. It can increase its efforts to reach all the groups working or interested in working on population research; it can emphasize its special interest in providing space where these investigators can report their findings and exchange ideas; it can point out again the importance of population research in achieving the objectives of the JOURNAL; finally, it can draw attention to the clear statement made in the first issue, that the JOURNAL would include articles on "studies of the natural history of such diseases (without which, as a base line, estimates of the value of specific therapeutic measures cannot be made); environmental, occupational, and nutritional factors contributing to chronic illness; epidemiology; rehabilitation; . . . institutions and home care; community impact and socio-economic problems . . . ."

In short, the JOURNAL is more than just a medium for the publication of papers. It can be a strong force in the furtherance of knowledge of the beginnings of chronic disease by an editorial policy specifically aimed at presenting and interpretating all aspects of the problem—the laboratory, the clinical, and the population.

ANTONIO CIOCCO
University of Pittsburgh
Pittsburgh 13, Pa.

And

PAUL M. DENSEN
625 Madison Ave.
New York 22, N. Y.

Book Reviews

DERMATOLOGY. By Donald M. Pillsbury, Walter O. Shelley, and Albert M. Kligman, Philadelphia, 1956, W. B. Saunders Company. Pp. 1331, with 564 illustrations. Price $20.00.

This skillfully wrought textbook of dermatology has something of value in it for every physician. It is an extraordinary volume in that the authors somehow have avoided making a "new" book by the time-honored method of rehashing old material. Further, they have done remarkably well to eliminate the perpetuation of those traditional errors so common among medical textbooks in general and dermatologic textbooks in particular. Here is a refreshing, dynamic approach to a specialty that, in American medical school training, has not received the attention it deserves. The authors point out that ". . . any general physician who is practicing in a temperate zone will find that some 7 to 15 per cent of his patients present themselves with a chief complaint of a disease affecting the skin." To which, this reviewer adds that less than 1 per cent of time spent in medical school is allotted to those diseases!

Dermatology should be required reading for deans of medical schools and for professors of medicine, particularly those who believe that the department of dermatology should be dissolved into the major discipline of internal medicine. The text is not a mere reflection of other people's work. It is in a sense a summation of the great contributions that have come from the Department of Dermatology of the University of Pennsylvania, where dermatology has been allowed to develop *sui generis*. It shows clearly the progress that can be made when restrictions are removed and responsible people in a specialty are given the reins.

This textbook is for the medical student also, for it presents dermatology in a logical, clear style. Gone are the cumbersome, lengthy terms that so long have plagued the student and annoyed the physician. In their place is the basic terminology of essential, modern dermatology. *Chickenpox* is *chickenpox*, and *herpes zoster* is simplified to *zoster* with the explanation: "This simplification is logical, because herpes simplex and zoster are caused by different viruses, have no immunologic relation, and vary widely in their clinical manifestations and courses, with the sole exception of grouping of the vesicular lesions."

Certain chapters within themselves are classic and apply to all branches of medicine. For example, probably no general textbook of medicine contains a more lucid chapter on principles of genetics and hereditary disorders. As much can be said for the chapter "Psychocutaneous Medicine" in which the opening paragraph contains the intriguing statement: "A change in attitude is now apparent in the addition to the question, 'What disease does this patient have?' to 'What kind of patient does this disease have?' "

The graduate student in dermatology may justly criticize the scanty histopathologic descriptions and the absence of long reference lists that are so useful when he is preparing for board examinations. On the other hand, he will find great solace in common-sense statements such as this remark concerning tuberculosis cutis: "Tradition emphasizes the diagnostic value of the so-called 'apple jelly' nodule, which may be exhibited when a glass slide is pressed over the surface of the lesion (diascopic examination). Classically trained students are commonly frustrated in a search for such nodules, for the simple reason that they are not present!"

The experienced dermatologist may find that the book is lacking in practical value when he is looking for quick support for a diagnosis in a rare case, for the indexing is incomplete and may give only one reference to a disease that actually is mentioned or discussed in several places. A close reader may find what appear to be different opinions on given subjects, especially between different chapters. These minor inconsistencies are attributable to the present-day practice of printing chapters as the authors complete them. Presumably the later chapters reflect the authors' best opinion on these points.

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Dermatology is a testimonial to the organizational ability of Pillsbury and to the internationally known research accomplishments of Shelley and Kligman. Acknowledgments are given to seventeen others for their important contributions to the book, and to a large number of individuals and institutions who contributed illustrations and charts. It is divided into five sections with fifty-two chapters and 1,331 pages.

Section I, "Applied Basic Principles in Diseases of the Skin," has thirteen chapters that include basic anatomy and pathology, physiology of the skin, and fundamentals of cutaneous mycology and bacteriology. Even these ordinarily dull subjects are brought to life by the forthright approach and clear style of writing. Especially commendable is the chapter on basic pathologic patterns, wherein are outlined the alterations in growth, function, and injury of the cells, appendages, and systems. The first ten chapters have boldface summaries that aid considerably in simplifying the difficult subject matter. Section II, "Basic Principles and Clinical Applications of Allergy and Hypersensitivity," comprises five chapters that do much to clarify, without oversimplifying, the present-day knowledge of dermatologic allergy. In Section III, "Principles of Diagnosis," the importance of the medical history in dermatologic patients is stressed. The photographs and illustrations in the chapters on types of skin lesions and on distribution patterns are well selected and technically excellent. Section IV, "Dermatologic Therapy," is as up to date as is the rest of the book. Dermatologists will applaud the sensible recommendation to use either U.S.P. or proprietary preparations, whichever are better or more easily available.

The last part of the book, Section V, "Cutaneous Medicine," comprises twenty-six chapters that cover the entire field of clinical dermatology. Notably absent among the chapter headings is the title "Syphilis," which is properly relegated to its place among the "Spirochetal Infections." In the last section are the classic contributions of Shelley and Kligman; an excellent chapter on disturbances of pigmentation; the two previously mentioned chapters on hereditary cutaneous disorders, and psychocutaneous medicine; and a good, much-needed chapter on industrial dermatoses.

This is a textbook of exceptional quality and is a major contribution to the field.

John R. Haserick, M.D.

DIABETES MELLITUS—Handbook for Physicians—by Howard F. Root, M.D. and Priscilla White, M.D., Pp. 338, Tables 33; indexed. New York-Landsberger Medical Books, Inc. Distributed by The Blakiston Division of the McGraw-Hill Book Co. Price $7.00.

If one agrees that it is desirable to have available for physicians unambiguous recipes for management of patients with certain disorders, then this small offering may well be the paradigm of such handbooks. The authors, of course, have for years been co-authors of the standard Joslin monograph, *Treatment of Diabetes Mellitus*, and their handbook might be expected to be a stripped-down version of the larger work. In fact, this is not exactly the case. The handbook is in its own right a readable, lucid exposition of one eminently successful method for the management of diabetes. Where there are important differences of opinion, this fact is generally indicated. Where there is well established and relevant fundamental information concerning pathology, physiology, or biochemistry, this is noted succinctly. Even the main issues of certain current controversies in fundamental areas are stated, although the interested reader will have to go elsewhere for a bibliography. All in all, this handbook succeeds in giving some insight into pathophysiologic mechanisms and clear descriptions of the detailed management of diabetes mellitus and its complications. One of the few changes in the text that one would recommend lies in the discussion of fluid and electrolyte therapy of diabetic acidosis. One would wish for a good deal more attention to the principles underlying such therapy, since thoughtless application of a rigid schedule may be disastrous. In particular, despite a number of specifically stated cautions, there is still some textual ambiguity about administration of potassium. It should be emphasized that despite potassium deficit, patients in diabetic coma may have hyperkalemia and must not receive intravenous potassium unless the concentration of potassium in serum is known or, if this cannot be determined, if electrocardiographic signs of hyperkalemia are absent. If the latter guide is all that is available, then potassium should be administered intravenously only with almost continuous monitoring by electrocardiography.

K. L. Zierler

WORLD TRENDS IN CARDIOLOGY: Volumes I—V.

I. CARDIOVASCULAR EPIDEMIOLOGY. Selected Papers from Second World Congress of Cardiology and 27th Annual Scientific Sessions of The American Heart Association held in Washington, D. C. Edited by Ancel Keys, Ph.D., and Paul D. White, M.D., Pp. 193, with 23 tables and 41 figures; indexed. New York, 1956, Hoeber-Harper. Price $4.75.

II. CARDIOVASCULAR SURGERY. Panel Discussions. Selected Papers from Second World Congress of Cardiology and 27th Annual Scientific Sessions of The American Heart Association held in Washington, D. C. Edited by Helen B. Taussig, M.D., and Arthur S. Cain, Jr., M.D. Pp. 61; indexed. New York, 1956, Hoeber-Harper. Price $2.00.

III. BLOOD VOLUME AND CONTRACTILE PROTEIN IN HEART MUSCLE. Selected Papers from Second World Congress of Cardiology and 27th Annual Scientific Sessions of The American Heart Association held in Washington, D. C. Edited by Arthur S. Cain, Jr., M.D. Pp. 131, with 8 tables and 41 figures; indexed. New York, 1956, Hoeber-Harper. Price $3.50.

IV. CARDIOVASCULAR DIAGNOSIS AND THERAPY. Selected Papers from Second World Congress of Cardiology and 27th Annual Scientific Sessions of The American Heart Association held in Washington, D. C. Edited by Arthur S. Cain, Jr., M.D. Pp. 95, with 5 tables and 8 figures; indexed. New York, 1956, Hoeber-Harper. Price $3.85.

V. INSTRUMENTAL METHODS IN CARDIAC DIAGNOSIS. Selected Papers from Second World Congress of Cardiology and 27th Annual Scientific Sessions of The American Heart Association held in Washington, D. C. Edited by Arthur S. Cain, Jr., M.D. Pp. 100, with 11 figures; indexed. New York, 1956, Hoeber-Harper. Price $3.85.

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The official program of the Second World Congress of Cardiology held in September, 1954, states that the Symposia, Panel Discussions, and certain Memorial Lectures for which no abstracts appeared, would be transcribed and published after the Congress. These five volumes represent fulfillment of this promise, with the notable exception of the panel on electrocardiography and vectorcardiography, which consists only of a discussion of two cases.

It is unfortunate that the title *World Trends in Cardiology* and the format of these volumes are each so pretentious. The unsuspecting and prospective buyer of these books (the total cost of the set is $17.95) will be the victim of several deceptions: first, these pages do not supply the concept suggested by this title; second, the material does not represent selections from all scientific papers presented at the meetings as the title on the cover would lead one to suppose, but is really a record of certain specific meetings held in the Main Auditorium during the Congress; and, third, the classification of material into 5 separate volumes will be confusing. Perhaps the most important objection of all is that this material is over two and one-half years old, a fact that is neatly hidden from the large print in the frontis pages of 4 volumes and is referred to only in the foreword of the first book. The historical value of the volumes is undisputed but it would have been more honest to indicate clearly what they contain. A single, humbler volume, more simply prepared and hence less expensive, with a title indicating these were the recorded symposia, panels, and lectures as promised, would have been of greater value, especially if accompanied by a good index. Their present form is hardly justified by their contents.

It is difficult to see which part of the medical public these 5 separate volumes are intended to serve and attract. Their individual titles are as follows: Volume I. *Cardiovascular Epidemiology*, Volume II. *Cardiovascular Surgery*, Volume III. *Blood Volume and Contractile Protein in Heart Muscle*, Volume IV. *Cardiovascular Diagnosis and Therapy*, Volume V. *Instrumental Methods in Cardiac Diagnosis*. Four of the five are edited by Doctor Arthur S. Cain, Jr.

Drs. Ancel Keys and Paul D. White together are editors of the first volume, which is the longest. The contributions are in great part related to atherosclerosis; a smaller and much less profound material relates to rheumatic fever and even less to other forms of cardiovascular disease. It is surprising to find what, from the table of contents, could be expected to be a paper, turn out in fact to be a few remarks of half a page or less in length (e.g., Congenital Heart Disease, Rheumatic Heart Disease in Sweden). A paper on the nature of fibrinoid, which those interested in rheumatic and related collagen diseases might be much interested in, is unaccountably also contained in this volume, instead of Volume IV where rheumatic disease is discussed.

The second volume on *Cardiovascular Surgery*, edited by Dr. Helen B. Taussig and Dr. Cain, unfortunately does not contain a word of her opinion or experience. Indeed the section on Congenital Heart Disease appears to be a highly regimented panel without individual interchange or discussion. Contrary to the title which indicates it will cover the medical aspects of the surgical treatment these are hardly mentioned, whereas indications for surgery comprise the great majority of the section. It is, however, very interesting to be able to compare the opinions from all over the medical world on patent ductus, tetralogy of Fallot, coarctation of the aorta, and pulmonic stenosis. The panel on surgical treatment, while providing only the briefest of comments from each of the experts, offers an opportunity to see how these can disagree. Nevertheless this panel too is interesting, although the opinions given are in some instances no longer held, another example of the dated nature of these volumes. The controlled cross circulation method of Lillehei is being discarded now, although in this volume it is presented with enthusiasm.

The third volume certainly contains two outstanding contributions, Burton's George E. Brown Memorial Lecture on "Control of the Peripheral Circulation" and Rushmer's on "Residual Volume of the Ventricles," but it is confusing to find them in this volume whose title is *Blood Volume and Contractile Protein in Heart Muscle*. On the other hand, the second half of this title is well presented in two papers by Mommaerts and Bing.

The fourth volume, *Cardiovascular Diagnosis and Therapy* is by and large the best. It offers summaries of opinion of several different experts on such important subjects for example, as rheumatic fever, pheochromocytoma, and treatment of hypertension, coronary heart disease, and shock in myocardial infarction.

The fifth volume (*Instrumental Methods in Cardiac Diagnosis*), with the notable exception of Burchell's paper on "Indicator Substances in the Diagnoses of Congenital Heart Disease" (material which has appeared elsewhere in the literature), could well have been omitted from the group. Diagnostic instrumentation is so slightly discussed and the papers are so brief and superficial that they have little value. Again, as in Volume II, the chairman of this panel (in this case Dr. André Cournand) is never quoted. This volume also contains two memorial lectures eulogizing Frank N. Wilson and Willem Einthoven, respectively, two case presentations delivered before a panel of electrocardiographic and vectorial experts in order to hear their readings of the tracings and loops, and lastly a panel discussion on ballistocardiography.

As an historical record of part of the Second World Congress this material is important. It has appeared too late, however. Furthermore, these volumes do not really present "world trends in cardiology" and are not of general interest to the medical student or the general practitioner. For the cardiologist who could not be in Washington in September, 1954, these recorded symposia, panels, and lectures would be interesting

M. Irené Ferrer